PROCEEDINGS.

VOL. XXVII. JUNE, 1930. No. 9.

Peiping Section.

Peiping Union Medical College, April 17, 1930.

5018

Duration of Electrical Systole ("Q-T" Interval) in Cardiac Failure.

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In the effort to find an objective criterion of heart failure, the "Q-T" intervals in the electrocardiograms of 155 patients with heart failure have been measured previous to the administration of any drug. The results of Li and Cheer[1] on 178 normal Chinese subjects were available for comparison. The accompanying table gives the data in summary form. "K" is a constant in the formula, $S = K\sqrt{C}$, in which "S" is the "Q-T" interval in seconds, and "C" the cycle length in seconds. "K" is therefore a measure of the deviation of the "Q-T" interval relative to the cycle length, and may indicate the presence or absence of disturbances in the dynamics of the heart.

It will be seen from the table that "K" is greatly increased in the presence of heart failure, irrespective of the etiology. In the complete paper the data will be given full statistical treatment. Further results to be described later indicate that "K" is frequently increased in patients with heart disease who have slight or practically no failure. If the "Q-T" interval of the electrocardiogram be taken to have a relation to the duration of ventricular systole, the increased

[1] Cheer, S. N., and Li, R. C., *Chinese J. Physiol.*, 1930, iv, No. 2.

TABLE I.

Average Values of "K" in Normal Individuals and Individuals with Heart Failure.

Subjects	Male		Female	
	No. cases	Average "K"	No. cases	Average "K"
Normal individuals	112	0.3743 ±0.00122	66	0.3877 ±0.00212
Rheumatic heart disease with failure	39	0.4227	28	0.4235
Syphilitic heart disease with failure	15	0.4400	2	0.4405
Hypertension and arteriosclerosis with failure	24	0.4405	24	0.4338
Miscellaneous heart disease with failure	16	0.4275	7	0.4320

"K" indicates a disturbance of cardiac dynamics which might well be found before clinical evidence of failure is available.

5019

Surface Tension of Egg Albumin Solutions.

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Although it is generally believed that egg albumin is a capillarilly active substance, contradictory statements are often found in literature. According to Clark and Mann[1] egg albumin increases the surface tension of water in very dilute solutions but lowers it in more concentrated solutions. It is impossible to draw any conclusion from the works of early investigators because most of them did not pay any attention to the reaction of the solution which is an important factor as shown by St. Johnston[2] and qualitatively by Bottazzi.[3] The latter found the minimum surface tension to be at the isoelectric point, while the former showed the reverse to be the case. The present study deals with the relation between surface tension and the hydrogen ion concentration of egg albumin solutions together with

[1] Clark, G. L., and Mann, W. A., *J. Biol. Chem.*, 1922, lii, 180.

[2] St. Johnston, J. H., *Biochem. J.*, 1927, xxi, 1314.

[3] Bottazzi, F., *Colloid Chemistry*, Edited by J. Alexander, Chemical Catalog Co., New York, 1928, Vol. ii, p. 121.

an approximate calculation of the cross-sectional area of the egg albumin molecule.

As we are interested in the equilibrium surface tension, the drop weight method as recommended by Harkins and Brown[4] was adopted, the only modification being that the liquid was pushed over instead of being sucked through the capillary. A necessary condition in this static method is that sufficient time must be allowed for the surface to reach equilibrium. It is well known from the work of Du Noüy[5] and others that the surface tension of a freshly prepared egg albumin solution decreases on standing to a constant value. For the most dilute solutions we used, more than an hour must be allowed to reach equilibrium, while with more concentrated solutions 15-20 minutes will suffice. Egg albumin was recrystallized 3 times from ammonium sulphate solution and dialyzed until no ammonia or sulphate could be detected. The stock albumin solution was sterilized by filtration through a Seitz filter. All solutions were made up with conductivity water under sterile conditions. The reaction was varied by addition of either HCl or NaOH and the pH values were determined electrometrically using a quinhydrone electrode. The amount of HCl or NaOH added was so small that its effect on surface tension may be neglected.[7]

We used egg albumin solutions ranging from 0.0000005 to 1%. Under no circumstance was the surface tension of the solution greater than that of pure water. At all concentrations the minimum surface tension was found at the isoelectric point, *viz.*, about pH 4.8. There are 2 maxima, one on the alkaline side and the other on the acid side of the isoelectric point. Four curves for the lower concentrations of albumin are shown in Fig. 1.

The difference between our results and those of St. Johnston may be explained by the fact that they used Sugden's method[6] which is essentially dynamic in nature, while the method we used is a static one. It has been pointed out by Price and Lewis[7] that the high surface tension observed at the isoelectric point is due to the absence on the particles of electric charge which lowers the surface tension. As the lowering of surface tension is due, in the first place, to the presence of egg albumin on the surface, we would expect the maximum lowering to occur at the isoelectric point, since at this reaction, on account of the absence of electric charge, more molecules or

[4] Harkins, W. D., and Brown, F. E., *J. Am. Chem. Soc.*, 1919, xli, 515.

[5] Du Noüy, P. L., *Surface Equilibria of Colloids*, Chemical Catalog Co., New York, 1926.

[6] Sudgen, S., *J. Chem. Soc.*, 1922, cxxi, 858; *Ibid.*, 1924, cxxv, 27.

[7] Price, H. I., and Lewis, W. C. M., *Biochem. J.*, 1929, xxiii, 1030.

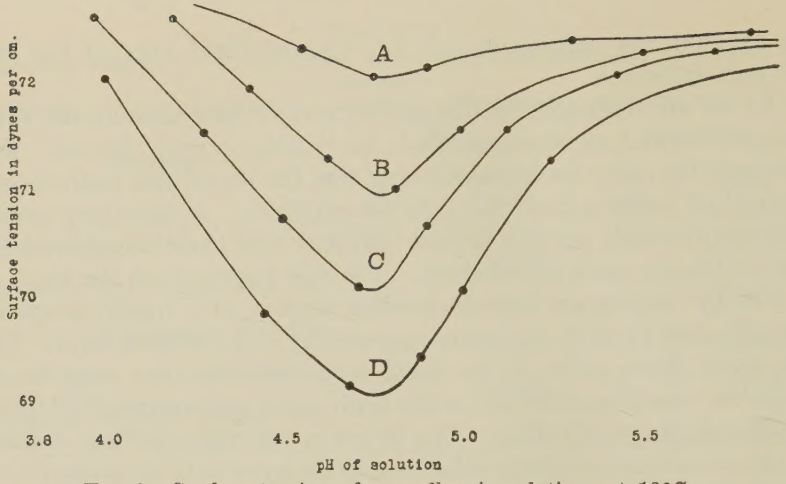

FIG. 1. Surface tension of egg albumin solutions at 18°C.
A. 0.0003%. B. 0.0006%. C. 0.001%. D. 0.002%.

molecular aggregates of egg albumin can be accommodated on a given surface if time is allowed for diffusion. In Sugden's method, the electric factor is more important than the concentration factor, because the molecules can not diffuse to the surface in the short time allowed for measurement, while in our case the surface concentration is more important than the electric factor.

Milner[8] has suggested a method for calculating the effective cross-sectional area of molecules from surface tension data. In Gibbs' adsorption equation,

$$u = -\frac{1}{RT} \cdot \frac{d\sigma}{d \log c}$$

the amount adsorbed per unit area u should be constant over a certain range of concentration if $d\sigma/d \log c$ is constant. This is true when the surface of the solution is covered by a single layer of molecules.

Knowing u the effective cross-sectional area a can be calculated by the relation $a = 1/uN$, where N is Avogadro's number. In our calculations concentrations instead of activities were used. This is justified as our solutions were so dilute that concentration and activity may be assumed to be identical. It is also assumed that the surface layer is monomolecular. As the concentrations are so low, it is not likely that the molecules adsorbed on the surface will pile up to form a multimolecular layer as suggested by McBain and Davis.[9]

[8] Milner, S. R., *Phil. Mag.*, 1907, xiii, 96.

[9] McBain, J. W., and Davis, G. P., *J. Am. Chem. Soc.*, 1927, xlix, 2230.

From the data shown in Fig. 1 we have plotted the minimum values of σ against log c and the curve obtained is a straight line with the slope of $d\sigma/d \log c = -1.59°$. It follows from this that

$$u = \frac{1.59}{8.31 \times 10^7 \times 291} = 6.56 \times 10^{-11} \text{ mols. per sq. cm.}$$

$$a = \frac{1}{6.56 \times 10^{-11} \times 6.1 \times 10^{23}} = 2.5 \times 10^{-14} \text{ sq. cm.}$$

5020

Blood Volume in Hyperthyroidism.

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It is a well recognized fact that many patients with hyperthyroidism have disturbances of their cardiovascular system. With the increased demand for oxygen some compensatory mechanism must be at work in order to supply the needs of the tissues. The cardiac output and the velocity of blood flow have been repeatedly shown to be increased, and as a rule, this increase is in direct proportion to the basal metabolic rate.[1, 2] In the opposite condition, myxedema, Thompson[3] described a reduction in the blood volume and demonstrated its return to normal after the institution of thyroid treatment. Blotner, Fitz and Murphy[4] also observed a correlation between the basal metabolic rate and the total blood count expressed in corpuscles per square meter of surface area. The present report deals with the blood volume findings in individuals suffering from hyperthyroidism, before and after subtotal thyroidectomy.

The series studied consisted of 17 cases of exophthalmic goiter and one of toxic adenoma.* The total blood volume was measured by the carbon monoxide method.[5] All the determinations were made

1 Fullerton, C. W., and Harrop, G. A., *Bull. Johns Hopkins Hosp.*, 1930, xlvi, 203.

2 Blumgart, H. L., and Gargle, S. L., *J. Clin. Invest.*, 1928-29, vi, 18, (Proceedings).

3 Thompson, W. O., *J. Clin. Invest.*, 1925-26, ii, 477.

4 Blotner, H., Fitz, R., and Murphy, W. P., *J. Clin. Invest.*, 1928, vi, 4, (Proceedings).

* The first seven cases were studied in the Johns Hopkins Hospital and are reported by courtesy of Dr. G. A. Harrop.

5 Chang, H. C., and Harrop, G. A., *J. Clin. Invest.*, 1928, v, 393.

TABLE I.
Blood Volume in Hyperthyroidism Before and After Subtotal Thyroidectomy in Successive Cases.

Before Thyroidectomy				After Thyroidectomy				Absolute Decrease				Percentage Decrease			
B.M.R. percent normal	Blood Volume			B.M.R. percent normal	Blood Volume			B.M.R. percent normal	Blood Volume			B.M.R.	Blood Volume		
	Total cc.	Per kilo cc.	Per sq. meter cc.		Total cc.	Per kilo cc.	Per sq. meter cc.		Total cc.	Per kilo cc.	Per sq. meter cc.		Total	Per kilo	Per sq. meter
155	3730	81.0	2590	90	3290	59.5	2125	65	440	21.5	465	41.9	11.8	26.5	21.9
131	3315	73.0	2240	85	2875	61.8	1920	46	440	11.2	310	35.1	13.3	15.3	13.8
151	3610	58.0	2090	102	3190	50.0	1823	49	420	8.0	267	32.5	11.6	13.8	12.8
134	5120	76.4	2910	94	4320	63.4	2440	40	800	13.0	370	29.9	15.6	17.0	16.2
173	4041	74.1	2580	107	3590	64.1	2272	66	451	10.0	308	38.2	11.2	13.5	11.9
133	3780	74.0	2860	86	3595	65.0	2300	47	1185	9.0	560	35.3	24.8	12.2	19.6
130	4880	69.0	2640	84	4120	61.0	2500	46	760	8.0	140	35.4	15.6	11.6	5.3
147	3100	62.4	2095	85	2875	56.8	1843	62	225	5.6	252	42.2	7.3	9.0	12.0
154	4552	86.3	2958	80	3170	62.0	2058	74	1382	24.3	900	48.9	30.4	28.2	30.4
168	3325	62.6	2190	104	2960	55.0	1936	64	365	7.6	254	38.1	11.0	12.1	11.6
143	3250	69.2	2258	80	3200	53.0	2000	63	50	16.2	258	44.0	1.5	23.4	11.4
174	4500	95.0	3100	105	3380	72.7	2365	69	1120	22.3	735	39.7	24.9	23.5	23.7
150	5100	81.0	2930	91	4555	75.5	2663	59	545	5.5	237	39.3	10.7	6.8	8.9
140	3285	78.0	2570	97	3245	77.3	2535	43	40	0.7	35	30.7	1.2	0.9	1.4

in the afternoon about 3 hours after the noon meal. Patients with fever and organic lesions of the heart were excluded. The results are expressed in cc. of blood per kg. of body weight. The minimum blood volume was 58 cc. per kg. and the maximum, 115 cc., with an average volume of 77.3 cc. The average total blood volume in normal individuals studied with the same method was 66.6 cc. (6)

The blood volume of 14 patients was again determined after their basal metabolism returned to normal following subtotal thyroidectomy. The time between the operation and the determination varied from 10 days to 5 months, and some patients had repeated measurements. On the basis of cc. of blood per kg. of body weight, all but one showed a striking fall in their circulatory volume, the decrease ranging between 6.8 and 28.2%. The percentage decrease in the total volume and in the volume per square meter of surface was approximately of the same magnitude. One patient was followed for a period of one year and 9 months. Her blood volume remained at the same level as that observed soon after operation, about 28% lower than the initial measurement.

Four patients were studied after the administration of Lugol's solution. This treatment seemed to have a definite effect in diminishing the blood volume along with the drop in basal metabolic rate. Following partial removal of the thyroid gland in these patients there was a further decrease in the blood volume.

5021

A New Form of Apparatus for Determination of Gases by Manometric Measurement.

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In the course of a certain study on hemoglobin it was necessary to mix thoroughly the solution to be analysed with the reagent before evacuation and extraction. As this could not be done in Van Slyke's apparatus,[1] we have devised a new form of the gas burette which, besides serving our special purpose, possesses certain advantages over the form now generally used.

The principal feature of the apparatus (Fig. 1) is that 2 cham-

[1] Van Slyke, D. D., and Neill, J. M., *J. Biol. Chem.*, 1924, lxi, 523.

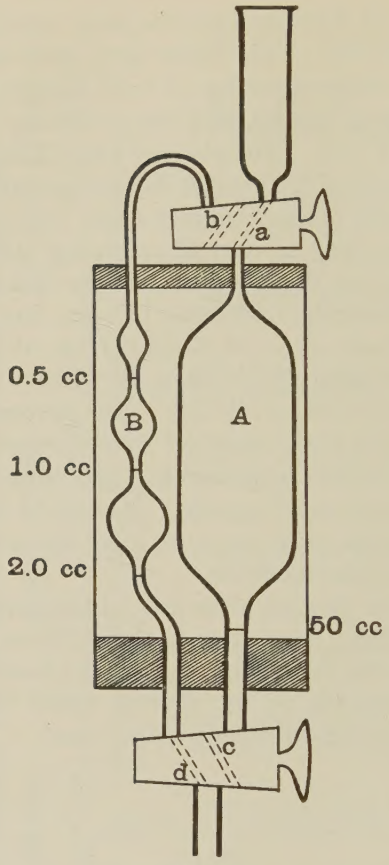

FIG. 1.

A new form of apparatus for determination of gases by manometric measurement.

bers are used instead of one. The extraction is carried out in one chamber (A) while the gas is measured in another (B).

The new apparatus is used essentially in the same way as Van Slyke's. The apparatus is first rinsed with H_2O, and both chambers, including the cock spaces, are completely filled with mercury. The solutions are then introduced into chamber A and the extraction is carried out as usual. On account of the high density of mercury it is desirable to evacuate chamber B also before shaking the apparatus. When the extraction is finished the upper cock is turned to connect A and B. If the pressure in A is high, the mercury in cock space b and above it is pushed over into B. If this does not happen when the cock is opened, shake the apparatus for a few seconds. Mercury is admitted into A and the solution is brought just to the top of A or to a mark slightly above the cock space b—according to how

the chamber B has been graduated. The lower cock is then turned to connect B to the manometer and the reading of the pressure is taken in the usual manner at 2, 1 or 0.5 cc.

By proper manipulation of the cocks and the levelling bulb, the gas is transferred back into A. The gas may now be ejected from this chamber or it may be absorbed with a reagent After the absorption the gas left is again transferred to B for measurement.

The advantages of the new apparatus over the usual form of Van Slyke's are as follows:

1. The gas dissolved in a solution may be completely extracted by repeated extraction in chamber A and storing the gas in chamber B. The total volume of gas thus extracted is measured in one reading. This advantage is important when the coefficient of absorption of the gas in the solvent is unknown.

2. Since no solution is admitted into the measuring chamber, it is always clean and there can be no error in volume measurement on account of precipitates adhering to the wall or on account of improper drainage of solution. Furthermore, the volume of the gas is measured between marks on a capillary tubing, and gas can be measured at 0.5 cc. with practically the same accuracy as at 2.0 cc.

3. The zero (vacuum) reading for the same kind of solution is constant, irrespective of the volume of solution used for extraction, which is not the case in the usual form of Van Slyke's apparatus.

4. In case more than one gas are to be determined by absorption with different reagents, the gas can be stored in chamber B while the solution is rejected from chamber A. If desired, the reagent can be made gas-free by extraction in chamber A. This can be cleaned if necessary after each absorption. Only a minimal amount of the reagent needs to be used and the error due to gases evolved from, or dissolved by, the reagent is thus reduced to the minimum.

5. Without evacuating the chamber, the solution and reagents can be thoroughly mixed in the chamber by gently shaking the apparatus.

5022

Preservation of Mitochondria in Fat Solvents by Uranium Nitrate.

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Attention has already been called to the fact[1] that Cajal's uranium-silver method, devised primarily to demonstrate the Golgi apparatus, occasionally stains mitochondria. We are not aware of any critical test on record made to determine whether or not this method, without the part of silver-nitrate impregnation, is adaptable to the demonstration of mitochondria. In the course of our studies on the effects of heavy metallic salts upon cell structures, we have had occasion to use Cajal's "uranium-formalin fluid" as a general fixative. Various tissues of frog, white mouse, and guinea-pig were fixed in this fluid for 10 hours. After a quick wash in distilled water, the pieces were dehydrated and embedded in paraffin, and sections were stained with fuchsin and methyl green. In all cases, it was found that the mitochondria had been well fixed and excellently differentiated but mostly in cells near the surface of the block, the deeper-lying ones being less affected. This was evidently due to the poor penetration of the fixative. An effort was made therefore to improve this effect by adding to the fixing fluid a small quantity of glacial acetic acid. The results were so satisfactory that this method seems to be in certain respects better than others so far available.

Further, we experimented on a mixture of this "uranium-formalin fluid" with fat solvents such as alcohol of 80% to 100% strength, ether and chloroform in various proportions with a view to testing their solubility upon mitochondria. It was surprising to find that in tissues, notably pancreas, liver, kidney, gastric and intestinal glands, the mitochondria remain either in long filaments or short rods, distributed abundantly in the superficial as well as in the deeper-lying cells. This observation shows that fat solvents such as acetic acid, alcohol, etc., when combined with uranium-formalin solution, do not dissolve the mitochondria. It seems probable that the mitochondria are rendered insoluble in fat solvents by the uranium salt, perhaps just as in the case of Zenker's fluid plus acetic acid where the bichromate may have oxidized the mitochondria so that they are no longer soluble in acid.[2]

[1] Da Fano, C., *Med. Sci.*, 1924, ii, 319.

[2] Young, R. T., *Anat. Rec.*, 1928, xl, 351.

5023

The Mydriatic Effect of a Mixture of Ephedrine and Homatropine.

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The use of a mixture of ephedrine and homatropine as a mydriatic for diagnostic purposes has been recommended by Geppert and Groenouw.[1] They found that a small amount of homatropine enhances the mydriatic effect of ephedrine, and the time at which mydriasis passes off is practically equal to ephedrine. Later this observation was repeated by many clinicians[2] using "Mydrin", which is a mixture of ephedrine and homatropine. By comparative experiments with ephedrine and homatropine we have further observed the mydriatic effect of this mixture.

White rabbits were used. The horizontal diameter of the pupil was measured in a dark-room, the source of light being at a constant distance. The change of pupil in strong light was also observed at a constant distance from a lamp. The drugs were applied to the conjunctival sac. A great number of preliminary experiments showed that the pupil on both sides responded to the drugs at practically the same rate and strength. The mixture of ephedrine and homatropine was in the proportion of one hundredth part of the latter to one part of the former substance.

In 6 experiments the mydriatic effect of a small dose of ephedrine on the homatropinized eye was observed. A small amount of homatropine such as one drop of 0.001% to 0.005% solution was applied to the eye on one side (which would produce a dilatation of 1 to 2.5 mm.) and half an hour later one drop of 0.1% to 1% ephedrine solution was applied to the homatropinized eye and also to the eye of the other side. In one case ephedrine (7 mg. per kilo) was injected intravenously. The homatropinized pupil was further dilated by ephedrine but its dilatation was simply due to an additional effect of the mydriatic action of ephedrine and homatropine and was not an augmentation of the homatropine effect.

In 11 experiments a mixture which was prescribed by Geppert and Groenouw for clinical purposes, was used. This mixture con-

[1] Geppert and Groenouw, *Dtsch. Med. Wochenschr.*, 1894, 161.

[2] Stephenson, S., *The Lancet*, 1898, ii, 24; Suker, *New York Med. J.*, 1895, 714; Cattaneo, *Clinica Moderna*, 1896, July; Snell, S., *Clinical J.*, 1895, Nov.; Sattler, C. H., *Klin. Monatsbl. f. Augenheilk.*, 1927, lxxix, 524, etc.

sisted of a watery solution of 10% of ephedrine and 0.1% of homatropine. One drop was placed on the eye of one side and its mydriatic effect was compared with one drop of 10% ephedrine solution (Fig. 1) or 0.1% homatropine solution (Fig. 2), which was applied to the eye of the other side in the same rabbit. In 5 cases the mydriatic effect of this mixture was compared with the effect of the subsequent addition of ephedrine of the same strength, applied after producing a maximal effect with one drop of 0.1% homatropine solution (Fig. 3). A further comparison was made of the effect of homatropine of the same strength applied after ephedrine (Fig. 4).

The results may be summarized as follows: 1. The duration of

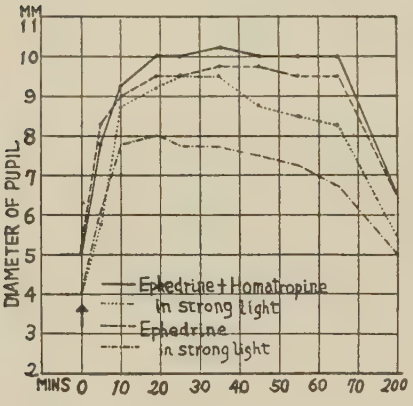

FIG. 1.

Comparison of the mydriatic effects of the mixture and ephedrine.

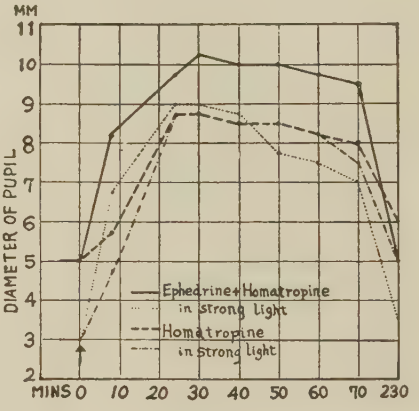

FIG. 2.

Comparison of the mydriatic effects of the mixture and homatropine.

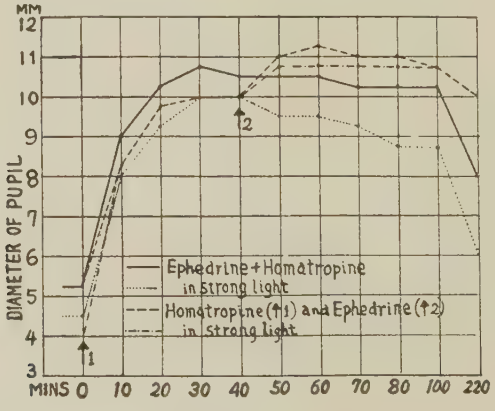

FIG. 3.

Comparison of the mydriatic effect of the mixture and the effect of ephedrine after homatropine.

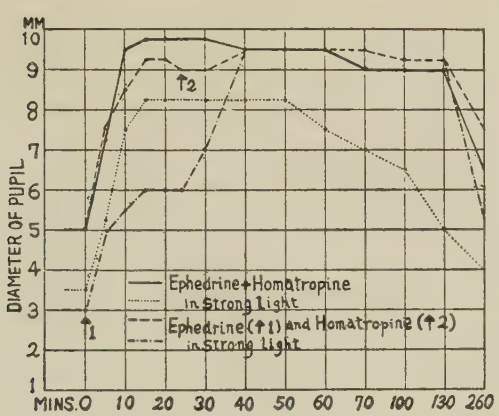

FIG. 4.

Comparison of the mydriatic effect of the mixture and the effect of homatropine after ephedrine.

the mydriatic effect of the mixture seems to be shorter than that obtained with homatropine and practically equal to ephedrine. 2. The mixture does not react to the light as much as ephedrine but is more reactive than homatropine. 3. The rate of dilatation of the pupil with the mixture is more rapid than with homatropine but at about the same rate as ephedrine. 4. The maximal dilatation with the mixture is greater than ephedrine or homatropine. 5. The 2 drugs given in sequence do not yield the same results as the mixture. Ephedrine given after producing a maximum effect with homatropine produces a further dilatation which is greater than that produced by the mixture; the light reflex abolished after homatropine is slightly recovered by adding ephedrine. Homatropine applied after ephedrine shows a dilatation similar to the mixture but the reaction to light is further greatly diminished. The mydriasis obtained by applying ephedrine and homatropine in sequence returns more slowly to the normal than the mixture.

5024

Demonstration of the Humoral Agent in Fat Inhibition of Gastric Secretion.

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Since the ingestion of fat results in the inhibition of the secretion of autotransplanted (*i. e.*, completely denervated) gastric pouches (Feng, Hou and Lim[1]), the mechanism concerned must be a humoral one. It was thought that as fat causes the ejection of bile into the duodenum, the reabsorbed bile (salts) might be the humoral agent, but this has been proved not to be the case (Kosaka and Lim[3]). The influence of fat on the gall bladder has been shown by Ivy and Oldberg[2] to be due also to a humoral agent. Having a sample of Ivy's gall bladder hormone, *viz.*, cystokinin, on hand, this was tried and found to inhibit gastric secretion in large doses (5 gm. per kg.). An attempt was therefore made to recover the gastric inhibitory agent from the intestine.

The upper small intestine of dogs (under ether or decerebrate) was ligatured in segments about 10 cm. long, and 50 cc. of olive oil introduced into alternate segments. After one hour, the intestine was removed and the mucosa exposed and unexposed to oil separately scraped off and extracted with N/10 HCl at a relatively low temperature (about 60°C.). Extracts have also been made simply with saline. Extracts from mucosa exposed to oil inhibit gastric secretion to a meat meal, when injected either intravenously or subcutaneously into Heidenhain-pouch dogs, the degree of inhibition produceable being comparable with that caused by the oral ingestion of fat (see Table I). Similar doses of the saline extract appear to have no effect on either the pancreatic or biliary secretion and do not depress the blood pressure. Further, when these extracts are heated to 80-100°C. for 10 minutes, the inhibitory effect is removed, and if HCl is added before heating, a gastric stimulating action is frequently obtained. Extracts from segments not exposed to oil exhibited no inhibitory action.

Extracts of the lower intestinal and colonic mucous membranes, which have been exposed to oil, are also inhibitory, while extracts of normal (*i. e.*, unexposed to oil) mucosa have no effect.

[1] Feng, T. P., Hou, H. C., and Lim, R. K. S., *Chinese J. Physiol.*, 1929, iii, 371.
[2] Ivy, A. C., and Oldberg, E., *Am. J. Physiol.*, 1928, lxxxvi, 599.
[3] Kosaka, T., and Lim, R. K. S., *Chinese J. Physiol.*, 1930, iv, 213.

TABLE I.

The inhibition of gastric secretion by enterogastrone and other comparative data.

No. of Obs.	Basal secretion before feeding		Procedure (150 gm. meat, 100 cc. water)	Active secretion after feeding			
	2nd hr.	1st hr.		1st	2nd	3rd	4th
30	4.3	6.7	Control (standard meal)	32.4	21.8	11.9	7.3
22	1.4	2.7	Enterogastrone (from intestinal or colonic segments exposed to oil)	9.5	10.0	7.3	5.6
9	3.6	4.2	Control extract (from segments not exposed to oil)	48.5	16.0	10.9	7.0
8	1.0	1.5	Enterogastrone heated to 80°-100°C.	34.3	14.6	8.0	5.4
4	1.0	1.2	Olive oil into colon (40 cc.)	4.2	4.7	5.2	3.8
2	0.9	0.7	Other substances into colon	27.2	12.7	9.4	4.9
14	2.8	4.5	Olive oil *per os* (50 cc.)	4.8	16.4	17.6	10.4

Introduction of 40 cc. of olive oil into the colon at the same time as feeding meat, inhibits gastric secretion, while the introduction of other substances, *e. g.,* saline, gastric content, in large quantity fails to do so.

Extracts of gastric mucous membrane after exposure to oil have no inhibitory effect.

These observations show that an inhibitory agent may be formed in the mucosa of both the small and large intestine as the result of contact with fat, and provide further evidence in substantiation of the humoral theory of inhibition. The name Entero-gastrone (derived from *entero/n, gastr/on* and *chal/one*) is suggested for the gastric inhibitory agent.

Southern Section.

Tulane University, May 24, 1930.

5025

Effects of Mercury Arc Radiation on Nutritional Anemia in Rats.*

PAUL C. FOSTER. (Introduced by Henry Laurens.)

From the Laboratory of Physiology, School of Medicine, Tulane University of Louisiana, New Orleans.

It was reported[1] earlier that white flaming carbon arc and quartz mercury vapor energy had little or no effect on a milk anemia in

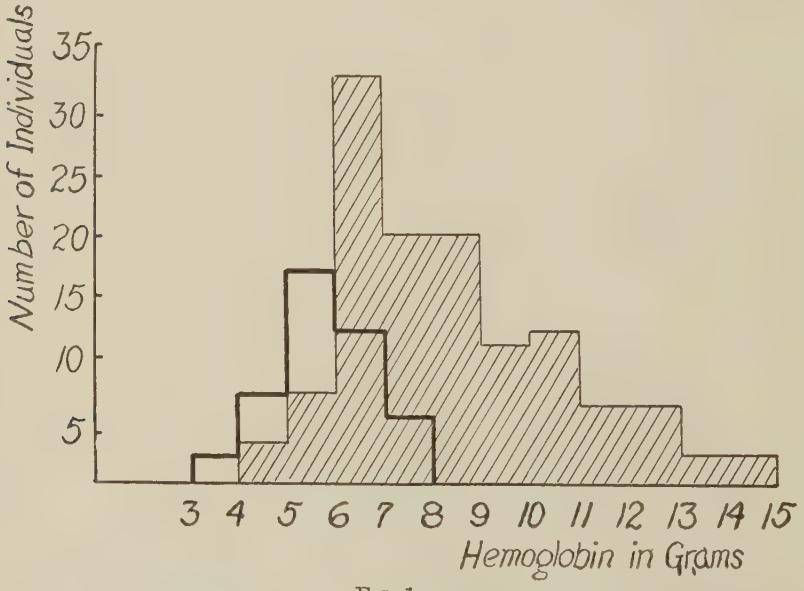


FIG. 1.
Frequency diagram showing the effect of 8 weeks' irradiation with the mercury arc on hemoglobin content. The area under the heavy lines is for non-irradiated, the shaded area for the irradiated animals.

* Aided by a grant from the David Trautman Schwartz Research Fund of Tulane University.

[1] Foster, P. C., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 751.

rats. Most of the observations were made with the carbon arc and continuation of the work has demonstrated a definite effect by the mercury arc, as evidenced by number and size of reds and hemoglobin content and saturation. Doses of about 10 minutes daily at a distance of 35 cm. seem optimal, larger doses showing a tendency to depress growth and produce burns. Doses of less than 5 minutes were practically without effect. Again no definite results have been obtained following exposure to the energy emitted by "Sunshine" carbons.

5026

Effects of Carbon and Mercury Arc Radiation on Acetylphenylhydrazine Anemia in Dogs.*

HENRY LAURENS AND H. S. MAYERSON.

From the Laboratory of Physiology, School of Medicine, Tulane University of Louisiana, New Orleans.

Anemia was produced in 18 adult dogs on a standard diet by subcutaneous injection of acetylphenylhydrazine (pyrodin). Eight animals were irradiated with the flaming carbon arc (Pan-Ray-Arc burning "Sunshine" carbons), 3 with the quartz mercury vapor lamp (Cooper-Hewitt), and the remainder served as controls.

Although there were variations in the reactions to pyrodin and to radiation the regeneration of the red cells was unquestionably faster in the irradiated group. The average count of the control group 23 days after the injection and 15 days after the lowest anemic level was 71% normal. The average value for the same number of irradiated animals that developed a comparable degree of anemia was 85% normal. The carbon and mercury arcs were equally effective. The difference in hemoglobin regeneration is not so marked, the average values 15 days after the lowest anemic level being 77% and 82% normal in the control and irradiated animals respectively. This relatively greater response of the red cells to irradiation was previously reported in studies on hemorrhagic anemia. No significant differences in reticulocytes, whites, platelets and red cell fragility were observed. The development of a tolerance to the drug on repeated injections invalidated attempts to use the same animal for control and irradiation periods.

* Aided by a grant from the Committee on Radiation of the National Research Council.

5027

A Fluoroscopic Study of the Motility of Gastro-Intestinal Tract of Rats Fed a Vitamin Deficient Diet.

LEON J. MENVILLE, J. N. ANÉ AND S. N. BLACKBERG.
(Introduced by W. H. Harris.)

From the Departments of Medicine and Pharmacology, Tulane University.

A review of the literature reveals that a vitamin deficient diet fed to animals will alter the motility of their gastro-intestinal tract, and is also capable of producing in their intestines marked pathological changes. Most of these observations appertaining to the motility, however, were made by methods other than with the Roentgen rays and in the few instances wherein it was employed, the skiagraphic method was used with unsatisfactory results.

In order that experimental observations upon animals may have a practical application to man, it is necessary to utilize animals whose diet is very much of the same nature as that of the human species, and their gastro-intestinal tract must bear a close resemblance to that of primates. In the rat we have an animal that will ideally serve this purpose, better in fact than herbivorous or graminivorous mammals.

A comparative study by means of frequent fluoroscopic observations was made on the motility of the gastro-intestinal tract of rats fed diets deficient in vitamin B, vitamin D and also a diet deficient in vitamins A and D with a balanced mineral content. These studies were undertaken principally to ascertain the effect of deficient vitamin A and D diet with adequate mineral balance, upon the motility of the gastro-intestinal tract of rats.

Seventy rats were used. Twelve were fed a deficient vitamin B diet, 14 a deficient vitamin D diet with an unbalanced mineral content, 17 a diet deficient in vitamins A and D but with an adequate mineral balance and 27 normal rats served as controls. After the rats were fasted for 48 hours and water withheld during the last 24 hours of fasting they were fed, in separate cages, a meal consisting of 5 gm. of barium sulphate and 5 cc. of buttermilk. They were allowed to eat for 20 minutes, when they were immediately fluoroscoped in loose cotton bags as previously described,[1] in order to ascertain whether their stomachs were full. Fluoroscopic observations were made every 15 minutes thereafter until the cecum ap-

[1] Menville, L. J., Blackberg, S. N., and Ané, J. N., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 758.

pearance of the food column was observed and continued until the small intestine emptied. The colon observations were made at longer intervals of time on account of the slow emptying of this organ.

The fluoroscopic observations made on the rats, demonstrated that those fed a diet deficient in vitamin B showed a marked hypomotility of their gastro-intestinal tract. The D deficient rats also showed a hypomotility, but not so marked as was found in the B rats. The A and D rats showed a motility comparable to the normal rats.

TABLE I.

Rats	Wt. Gm.	Ate Gm.	Cecum Appear. Time	Stom. Emp. Time	Sm. I. Emp. Time	Col. Emp. Time	No. of Rats
A-D	91.7	7.8	3:41	6:22	10:19	70 hr.	17
B	89.8	7.23	4:17	11:14	15:27	121.8 hr.	12
D	103.1	8.3	2:22	9:50	12:05	95 hr.	14
Norm.	184.0	6.3	3:19	7:04	10:36	65 hr.	27

Our table demonstrates by comparison the various emptying times of the gastro-intestinal tracts of rats fed diets deficient in certain vitamins. It can also be seen that the rats fed a deficient vitamin A and D diet show a striking similarity in their motility to that of the normal rats.

While we have made no attempt to prove in these experiments that a diet deficient in vitamin D alone produces a hypomotility of the gastro-intestinal tract, we believe, however, that it plays an important part, as the mineral content of the diet fed the A and D rats contained an adequate mineral balance, with a resulting motility comparable to the normal. This would indicate that the hypermotility produced by the deficient A vitamin as observed by many investigators was balanced by a factor antagonistic to it, and probably that a hypomotility was produced by a deficient D vitamin.

5028

Normal Intraventricular Conduction and Intraventricular Block Occurring in Adjoining Complexes.

GEORGE HERRMANN.

From the Heart Station, Charity Hospital, and the Department of Medicine, Graduate School of Medicine, Tulane University of Louisiana, New Orleans.

The comparative permanence of electrocardiographic abnormalities, especially bundle branch block, once established is an admitted maxim. The more promising possibility of the transient nature of such disturbances is usually not considered. In experimental work on conduction in the heart of the dog, Wilson and I[1] were impressed by the fact that relatively light pressure exerted over one of the main branches of the His' bundle would produce bundle branch block from which there was complete recovery within a few minutes and a return to an absolutely normal intraventricular conduction time which was recorded.

Lewis had observed a patient with transient bundle branch block as early as 1913. There have been 9 other instances recorded in the literature which at one examination presented defective conduction while at another later examination the normal intraventricular conduction was recorded and vice versa. In none of these instances, however, was there any record of a transition of a sudden nature. In fact, in most of them there is a suggestion of a gradual transition. This condition is not so uncommon since 5 similar cases have come under my observation.

Three additional cases of unusual interest and importance were encountered. These were extraordinarily unique in that the sudden transitions were recorded electrocardiographically. In each one of these instances the transition was within one beat and was from complete bundle branch block to absolutely normal intraventricular conduction. These observations constitute clinical corroboration of our experimental findings and of the pathological findings of Cohn and Lewis.[2] There seems to be evidence sufficient that temporary mechanisms and functional changes in the conductive system in the diseased human heart as well as in the normal dog heart may induce bundle branch block.

These 3 cases with the transition between bundle branch block and normal intraventricular conduction taking place within one beat

[1] Wilson, F. N., and Herrmann, G. R., *Heart* (London), 1921, viii, 229.

[2] Cohn, A. E., and Lewis, T., *Trans. N. Y. Path. Soc.*, 1914, xiv, 207.

seem to be proof positive that the disturbance was not of an organic nature, at any rate not totally so. It is quite conceivable that pathological changes may be present in the region of the bundle and primary branches which would require very little additional change for the precipitation of block. Under such conditions, however, one should expect to find slight transition periods and increasing block. A mechanical factor could quite conceivably add the necessary additional sudden and temporary factor. That there is a mechanical factor playing a part is substantiated by the fact that the periods of normal conduction in these patients were induced by respiratory maneuvers. The Müller's or Valsalva experiments, either of which were accompanied by the sudden conspicuous changes in intraventricular conduction, were apparently concurrent with the cardiac dilatation.

The clinical value of the observations lies in the realization of the fact that the serious prognostic significance of permanently organically produced bundle branch block is not to be applied until the possibility of a temporary disturbance is ruled out. Furthermore, as generally recognized, patients with bundle branch block are known to die suddenly at the slightest provocation, especially during a minor therapeutic or surgical procedure. Any blood pressure may be enough to press the already over exerting heart to a point of causing ventricular fibrillation and exitus. During the periods of normal conduction these patients are relatively good surgical risks. In one of our patients the discovery of the transient nature of the condition led us to follow her carefully electrocardiographically and to subject her to 2 successful surgical operations, a Cesarean and a later sterilization, during periods in which she was not blocking, without precipitating any evidence of undue cardiac embarrassment.

5029

Subarachnoid Spinal Adhesions: An Attempt Experimentally to Produce and Prevent Their Recurrence.

GILBERT C. ANDERSON AND ALTON OCHSNER.

From the Department of Surgery, Tulane University School of Medicine.

Subarachnoid spinal adhesions may at times produce clinical symptoms. Such a case has been observed by us in which following a laminectomy for paraplegia numerous adhesions of the spinal subarachnoid space were found. These adhesions were divided, but

as adhesions involving all serous membranes are apt to recur, the possibility of a recurrence of these subarachnoid adhesions was considered. As Herrmann and Musser,[1] and Ochsner and Herrmann[2] had successfully prevented the recurrence of adhesions in the pericardial cavity and Ochsner and Mason[3] in the peritoneal cavity experimentally by the use of digestants, it was thought possible that such procedures might be employed in the prevention of the recurrence of spinal subarachnoid adhesions.

Our purpose in this study was to produce subarachnoid adhesions by introducing an irritant into the spinal subarachnoid space in the lower thoracic region of dogs, later to divide these adhesions, and to attempt to prevent their reformation by introducing a digestion solution. We have been able to find no record of a previous attempt of this kind, although Dr. E. R. Schmidt, of the University of Wisconsin, has employed a digestant in the subarachnoid space in one case in order to attempt to prevent the reformation of adhesions.

The experimental animals employed were dogs. An attempt was made to inject the irritant into the spinal subarachnoid space by spinal puncture, but because of the difficulty encountered in performing a lumbar puncture, this method was abandoned. A formal laminectomy was employed in the remainder of the animals, and the irritant was injected into the subarachnoid space by means of a syringe and cannula. The animals were prepared in the usual manner; ether was used as the anesthetic agent. Following the injection of the irritant the wounds were closed in layers without drainage. Twenty-six dogs were operated upon; 6 died within 3 days and 7 of the remaining 20 died within 4 weeks. The 7 animals dying within 4 weeks all showed evidence of a paraplegia following operation and rapidly developed decubitus ulcers associated with marked emaciation. At autopsy in this group of animals an advanced myelitis at the site of operation was present in each case, and the spinal cord was so liquefied that no specimen could be obtained for microscopic study. No gross adhesions were demonstrable.

The 13 additional animals survived. Although 1 animal died within a week, it is reported in detail because in this particular animal a second operation was performed.

Protocol. Dog A1-3. August 7, 1928, 0.5 cc. aleuronat solution

[1] Herrmann, George, and Musser, J. H., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 314.

[2] Ochsner, Alton, and Herrmann, George, *Arch. Surg.*, 1929, xviii, 365.

[3] Ochsner, Alton, and Mason, Frank, PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 524.

injected within the dura, the lower dorsal region, following a laminectomy performed under ether anesthesia.

August 8—Animal is paraplegic.

From August 7 to August 14 the animal lost weight rapidly and became emaciated.

August 14—Operation under ether anesthesia. Previous laminectomy wound is opened under sterile precautions. The dura is opened, and numerous adhesions are found involving the subarachnoid space. These adhesions are divided and 0.5 cc. of a 1:1000 solution of papain is placed within the dura. The dura is, however, so friable that a satisfactory closure is not possible. The wound is closed. The animal died that night.

The remaining 12 animals survived for periods varying from 7 to 51 weeks, an average of 23.5 weeks. Six were paraplegic and showed evidence of myelitis at autopsy; 6 were not paraplegic and showed no evidence of myelitis at autopsy. Those which survived the longest were in general the ones that were not paraplegic, although 1 paraplegic animal survived for 28 weeks. The average survival for the paraplegic animals was 19 and for the non-paraplegic animals, 28 weeks. One of this latter group was killed in a fight when apparently in good condition. The amount of aleuronat solution varied from 0.1 cc. to 1 cc. Of the 6 paraplegic animals, 2 received 0.1 cc., 2 received 0.3 cc., 1 received 0.4 cc., and 1 received 1 cc. Of the 6 non-paraplegic 2 received 0.1 cc. of aleuronat, 3 received 0.3 cc. of aleuronat, and 1 received 0.5 cc. of aleuronat. None of the dogs that died early showed any adhesions except the one reported in detail, AL-3, and in this one there was a slight infection of the wound which may have been responsible for the adhesions. Of the 12 dogs which survived more than 4 weeks, 2 had very fine adhesions, whereas 9 showed no adhesions. In one animal, AL-4, at autopsy no adhesions in general were found, but along the line of incision the cord was firmly adherent to the overlying muscle. The dura in this particular animal could not be identified. This animal had been paraplegic.

Microscopic examination of the sections from this group of 12 animals did not show any evidence of adhesions. All animals showed some paresis following operation, but in those reported as non-paraplegic the paresis cleared up in varying degrees. In a few a moderate degree of paresis persisted, but in the majority there was apparently a complete recovery.

No valuable conclusions can be drawn from this experiment. It appears dogs do not tolerate foreign substances within the sub-

arachnoid space. Following the injection of an irritative substance into the subarachnoid space evidence of damage to the cord was seen in all animals and in only 6 was there recovery to such an extent that the use of the lower extremities returned. Only 4 animals developed adhesions and in 2 of these adhesions were scant and extremely fine. The failure to produce satisfactory adhesions prevented the performance of the second part of the experiment; *i. e.*, the prevention of the reformation of adhesions.

The occurrence of myelitis in all animals may have been due (1) to mechanical pressure and (2) to chemical irritation. We are inclined to accept the latter view (1) because of the extreme degree of cord destruction of those animals that died early and (2) because there was little clinical difference between the animals which received large and small amounts of aleuronat.

5030

Experimental Polyneuritis in Chickens Given Jamaica Ginger.

J. H. WATKINS. (Introduced by J. H. Musser.)

From the Department of Medicine, Tulane University.

Four apparently healthy chickens weighing approximately one kilo each were used in this experiment. Jamaica ginger obtained from a community where there were many patients with peripheral neuritis was used. Two of the chickens received daily doses of 2 cc. fluid extract of ginger and the other 2 received an equal amount of 83% ethyl alcohol. The chickens were kept in a cage out of doors and their diet consisted of cracked corn and oats. The chickens receiving the alcohol were accidentally killed 10 days after beginning the experiment.

Thirty-eight days after receiving the first dose of ginger and after each chicken had been given a total of 70 cc. there were no signs of muscular weakness. There had been some loss of weight during this time. On the thirty-ninth day the chickens exhibited slight motor weakness and loss of coordinating power in both extremities. Feeding ginger was discontinued at this time. During the next 2 days there was a progressive motor weakness and the difficulty in walking or standing was marked. Control of the feet was completely lost and the toes were turned under the feet when an attempt was made to walk. The legs rather than the feet were used

to support the body when resting. There was apparently no sensory disturbance of the extremities and no edema was observed.

A thick suspension of rice polishings was given through a tube when the paralysis was first observed and daily thereafter for a period of 5 days without any improvement. One of the chickens died 8 days after the onset of the paralysis. Sections of the nerve trunks have not yet been examined. Motion pictures were made to demonstrate the paralysis.

Further investigation is under way at the present time using a larger number of chickens and employing ginger with phenol, ginger without phenol, and ethyl alcohol. At the present time only those chickens receiving phenol ginger show leg weakness, 9 days after beginning the experiment.

5031

A New Method of Increasing the Precipitating Action of Syphilitic Serum.

FOSTER M. JOHNS.

The practice of heating serum to 56°C. for 30 or more minutes unquestionably increases the precipitating ability of blood serum from syphilitics for the saline suspensions of lipoid-cholesterin antigens now used in the precipitin test for syphilis. Insistence upon the use of "inactivated" serum in such precipitin tests apparently began in 1918 by Sachs and Georgi,[1] and was followed by Meinicke in his third modification in 1919,[2] and by Dreyer and Ward[3] in 1921, and Kahn[4] in 1922.

It is rather paradoxical that heating of serum should lower the "specific" antibody titre utilized in the Wassermann reaction by 75%, as shown in the now classical researches of Noguchi,[5] and simultaneously increase the precipitin titre by 8 times (from 10 to 80 Kahn units) as was recently shown by Kurtz[6] in Kahn's laboratory. The explanation advanced by Dreyer[3] of a complex anti-syphilitic antibody, composed of a thermostabile precipitin coupled

1 Sachs and Georgi, *Med. Klin.*, 1918, xiv.

2 Meinicke, E., *Munchen. Med. Wchnschr.*, 1919, xxxiii.

3 Dreyer, G., and Ward, H. K., *Lancet*, 1921, i, 956.

4 Kahn, R. L., *Archiv. of Dermat. and Syph.*, 1922, v.

5 Noguchi, H., *Serum Diagnosis of Syphilis*, J. B. Lippincott Co., Phil., 1910.

6 Kurtz, M. B., *J. Lab. and Clin. Med.*, 1930, xv, 7.

to a thermolabile substance which inhibits precipitins and favors complement fixation, is rather theoretical to say the least.

Kurtz quotes an apparently unpublished opinion by Nishio, a worker in Kahn's laboratory, "that the function of heating is to reduce the protective properties of the serum albumins to precipitation." This is a most plausible theory. By advancing another purely physical method of increasing the titre of the "specific" precipitin in serum the additional evidence may be sufficient to transfer this phenomenon to terms of a simple biochemical reaction.

Using my modification[7] of Butler's antigen[8] for the slide precipitation test for syphilis I have found that by allowing the serum to dry completely the precipitating action of syphilitic serum is increased to almost identically the same extent as when heated.

Pooled syphilitic serum was diluted with negative serum until a negative reaction was obtained with 0.15 cc. of the raw serum. A portion of this was inactivated at 56°C. for 30 minutes. Serial dilutions of both were made with normal saline, and 0.15 cc. of each dilution was placed on slides. When to be dried, the serum was spread over the middle third of the slide.

The antigen used for the dried serum was a 1 to 5 dilution of the following stock solution: Alcohol extract (1 to 10) of acetone defatted dried heart powder, 90 cc.; ethyl alcohol (98%), 10 cc.; 10% alc. sol. of repurified balsam of tolu, 4.5 cc.; 1% alc. sol. of benzoic acid, 1.25 cc.; cholesterin, 0.56 gm. For the plain and inactive serum a 1-4 dilution with normal saline.

0.2 cc. of the antigen suspension was added to each slide, mixing the antigen and serum immediately with a tooth pick. Readings were made at end of 5 minutes, during which time an occasional agitation of the mixture on the slides was made by tilting from side to side.

The results of a single experiment were as follows:

TABLE I.

Raw Serum	Heated Serum	Dry Serum	Serum Dilution	Fraction of 1 unit of (—) serum
No. ppt	Comp. ppt.	Comp. ppt.	None	1
,, ,,	,, ,,	,, ,,	1-1	½
,, ,,	,, ,,	,, ,,	1-2	⅓
,, ,,	,, ,,	,, ,,	1-3	¼
,, ,,	Partial	Partial	1-4	⅕
,, ,,	Slight	Partial	1-5	⅙
,, ,,	None	None	Normal saline control	

[7] Johns, F. M., *Proc. La. State Med. Soc., N. O. Med. and Surg. J.*, 1930.

[8] Butler, H. W., *N. O. Med. and Surg. J.*, 1928, lxxxi, 15.

Serum albumin acting as a hydrated colloid, as pointed out so beautifully by Fischer,[9] is able to form a fairly stable (emulsion) with any of the very delicately balanced precipitating systems used in serologic work. The slight upset to this balance of this system by partial coagulation of the albumin by heat (sufficient to produce a change in clarity when serum is properly inactivated) or the simple dehydration of the albumin by drying and then redissolving it removes the inhibiting effect to such an extent as to allow the full action of the precipitinogen. It is very doubtful if this "redissolved albumin" is as well hydrated or can function as well as it could in its natural state.

5032

CO_2 Combining Power of the Blood Plasma Before and After Ethylene Anesthesia in Diabetics Protected With Insulin.

I. I. LEMANN. (Introduced by J. T. Halsey.)

From the Department of Medicine, Tulane University, New Orleans, La.

Formerly diabetics were regarded as very poor surgical risks. Not only was there fear of infection and poor healing but the effect of a general anesthetic was dreaded. Since the advent of insulin it has been shown that diabetics may be operated upon with almost the same degree of safety as normal individuals. Even in normal persons the administration of general anesthetics may produce an acidosis. The metabolism may be profoundly affected. The post-operative period of vomiting and starvation contribute still further to the threat of acidosis. The administration of a general anesthetic to a diabetic, therefore, has been in the past a matter of no small concern. In spite of this there have been apparently recorded in the literature few definite observations as to the alkali reserve of the blood in diabetics under anesthetics and afterwards. The only 2 references I have been able to find in the insulin era have been those of Rabinowitch[1] and McKittrick and Root.[2] The former gives data as to the pH and CO_2 combining power in 2 diabetics under ether. He does not state whether insulin was used. McKittrick and Root state:

[9] Fischer, M. H., and Hooker, M. O., *Fats and Fatty Degeneration*, John Wiley & Sons, New York, 1917.

[1] Rabinowitch, I. M., *Current Researches in Anesthesia and Analgesia*, 1925, iv, 267.

[2] McKittrick, L. S., and Root, H. F., *Diabetic Surgery*, Lea & Febiger, 1928, 78.

"We have used the plasma CO_2 combining power as the best index of acidosis and it is true in 15 cases a reduction in this value occurred as a result of operation, with a return to normal within 24 hours due to the use of insulin and carbohydrate food." Rabinowitch pointed out: "The finding of a low CO_2 combining power in the plasma does not always necessarily denote acidosis. The lowered CO_2 combining power may be due merely to the increased pulmonary ventilation accompanying anesthetics. In increased pulmonary ventilation an excessive amount of carbonic acid is taken from the plasma and alkali passes into the tissues to maintain the $H_2CO_3/NaHCO_3$ ratio constant."

I have thought it worth while, therefore, to report the observations made on 17 diabetics to whom a general anesthetic was administered.

TABLE I.

Age	Operation	Anesthetic. Duration of Anesthesia	Pre-operative		Post-operative*	
			Blood sugar	CO_2	Blood sugar	CO_2
63	Gangrene of foot	Ethylene 45 minutes	133	50.4	148	50.4 (2 hr.)
73	Gangrene of foot	Ethylene 30 minutes	182	80.5	105	54.1 (1 hr.)
63	Gangrene of leg	Ethylene 65 minutes	117	51.3	110	53.2 (2½ hr.)
73	Gangrene of leg	Ethylene 45 minutes	103	53.2	133	48.6 (2 hr.)
60	Tonsillectomy	Ethylene 1 hour	80	50.4	200	46.7 (1¼ hr.)
52	Appendectomy	Ethylene	60	54.0	190	50.0 (2 hr.)
20	Mastoidectomy	Ethylene 30 minutes	71	56.0	76	57.9 (2 hr.)
50	Incision of finger abscess	Ethylene 4 minutes	105	48.0	117	53.2 (2 hr.)
59	Gangrene of leg	Ethylene 1 hr. 45 min.	286	35.3	238	35.3 (1 hr.)
62	Lap. for carcinoma of pancreas	Ethylene 30 minutes	190	43.8	200	45.3 (1½ hr.)
58	Hysterectomy for carcinoma of uterus	Ethylene 40 minutes	210	50.4	444	37.2 (2½ hr.)
16	Induction of labor at 5½ mos.	Ethylene	100	35.6	91	40.3 (1 hr.)
58	Cholecystotomy	Ethylene 68 minutes	83		172	31.5 (immed.)
55	Nephrectomy	Ethylene 2 hrs. 20 min.	110	52.0	400	42.0 (1½ hr.)
73	Gangrene of foot	Gas-ether	296	39.0	332	76.0 (1 day)
52	Mastoidectomy	Ether-oxygen	74	50.4	200	45.8 (3 hr.)
51	Removal of breast	Nitrous oxide 32 minutes	211	54.0	400	43.8 (1 day)

* Time given in this column = time post-operative at which blood specimen was taken.

Fourteen were given ethylene gas, 2 ether, and 1 nitrous oxide and oxygen. The tables will show the CO_2 combining power taken before the administration of the anesthetic and at various times subsequent to its cessation, usually from one hour to 2 hours after completion of the operation. The patients were prepared for the operation wherever possible by appropriate diet and insulin so that they would come to the operation approximately as normal individuals without ketosis and hyperglycemia. This was not in every case possible and it will be observed that in a number of instances the blood sugar was abnormally high at the time of the operation. The method used included the administration of an extra carbohydrate feeding and insulin dose about 6 or 8 hours before the operation and during the course of the anesthetic the administration of 20 cc. of 50% glucose intravenously with from one to 2 units of insulin per gram of glucose. The insulin was given hypodermatically. It will be observed that usually the general anesthetic caused no change in the level of the CO_2 combining power. This corresponded to the clinical picture for there was no evidence of acidosis. It will be observed also that the level of the blood sugar at the time of the anesthesia was immaterial. Equally good results were obtained with high blood sugar levels as with low. The pH was not determined.

5033

Experiments on the Effect of Di-hydranol on Intestinal Protozoa of Man and Laboratory Mammals.

ERNEST CARROLL FAUST.

From the Parasitology Laboratory, Department of Tropical Medicine, Tulane University, New Orleans, La.

Leonard and his associates (Leonard,[1] Leonard and Frobisher,[2] Hampil[3]) have demonstrated the bactericidal properties of the acyl and alkyl derivatives of resorcinol and Ratcliffe[4] has shown that the administration of certain of these derivatives to rats not only changes the intestinal flora from a Gram-positive to a Gram-negative type but also is effective in reducing the protozoa in the cecum

[1] Leonard, V., *J. Urol.*, 1924, xii, 585.

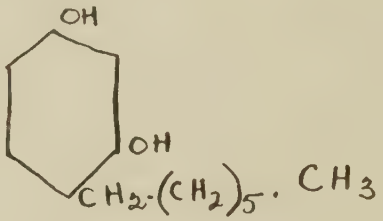
[2] Leonard, V., and Frobisher, M., *J. Urol.*, 1926, xv, 1.

[3] Hampil, B., *J. Inf. Dis.*, 1928, xliii, 25.

[4] Ratcliffe, Herbert L., *Am. J. Hyg.*, 1929, x, 643.

(Trichomonas spp., *Endamoeba muris).* Trichomonads in young chicks were also killed on a one per cent N-heptyl resorcinol diet fed for 5 days (Ratcliffe, l. c.). At the suggestion of Dr. Veader Leonard the writer has made a study of the effect of di-hydranol, a homolog of hexyl resorcinol, on certain intestinal protozoa of man and mammals in New Orleans. The drug has been prepared by Sharp and Dohme of Baltimore and been placed at the writer's disposal through the courtesy of Dr. Leonard.

Structure and properties of di-hydranol. Di-hydranol (2-4-dihydroxy-phenyl n-heptane) is a yellowish crystalline product with the structural formula:



Its taste is at first slightly sweetish, but soon there develops a definite mild acrid pungency, very similar to that of the root of the Indian turnip, *Arisaema atrorubens,* to be followed later by a mild numbness of the tongue. The drug is only slightly soluble in water. Hampil (l. c.) found the series to which this group belongs to be more bactericidal in alkaline solutions, presumably due to their greater solubility in this medium. In concentrated crystalline form it is too irritating for the human stomach but in olive oil, in which it is highly soluble, this difficulty is entirely obviated. Almost all of the drug is excreted by the bowel; its presence in the urine of a patient taking the drug in olive oil can be detected only by the most delicate technic. Its toxicity is clinically negligible.

Clinical material for study. Through the cooperation of several physicians 21 patients harboring intestinal protozoa have been available for study of the protozoacidal properties of the drug. These have consisted of 8 children from 2 to 9 years of age, 10 adolescent boys and 3 men. The protozoa which they harbored before treatment, together with the number of cases, are respectively as follows: *Endamoeba histolytica,* 14; *E. coli,* 9; *Iodamoeba buetschlii, 2*; *Endolimax nana, 2*; *Giardia lamblia,* 11. In addition, *Endamoeba histolytica* and *Giardia* have been treated in 2 dogs and 3 rhesus monkeys, *Giardia* in 3 rats, *Trichomonas* in 2 monkeys, and *Chilomastix* in 3 monkeys.

The dosage. One teaspoonful of olive oil, containing 0.2 gm. of di-hydranol, has been given to children 3 times a day after meals for a period of 7 days or more. Two capsules (1.2 cc.), containing 0.3 gm., have been administered to adolescents and adults 2 or 3 times a day after meals. In dogs and monkeys the compound (10 cc.) has been given once a day by duodenal tube; in rats one per cent of the crystalline drug was added to a balanced diet. In no instance except in the rats was it possible to have complete control of the diet. A total of from 4 to 10 gm. was given to each host studied. In every case the drug was well tolerated and there were no symptoms except in the case of 2 children, to which it had been administered before meals, when slight gastric pain was noted, and in 2 cases (adults) in which a slight diarrhea developed for 2 days.

Results of treatment. Follow-up microscopic examinations together with case histories provide the following data. In a total of 19 cases of *E. histolytica,* including 2 acute infections (dogs), one chronic case of 5 years' standing and refractory to other drugs (adult), 2 chronic (children), and 14 carrier cases, all except one carrier case have been freed of the infection with a single treatment series (4 to 10 gm.). Two of the 12 *E. coli* infections have been eliminated. The 2 *Iodamoeba* and 2 *E. nana* infections have been removed. The 2 *Trichomonas* infections (monkeys) have been eliminated. One of the 3 *Chilomastix* and 4 of the 14 *Giardia* cases (2 human, 2 canine) have been eliminated. The rats were completely freed of their *Giardia* infection on the third day of treatment, but gradually showed *Giardia* cysts in their stools. The question of reinfection from their cages is a possible explanation for this recurrence.

On the whole there are ample data to support the view that di-hydranol has a specific action on *Endamoeba histolytica, Iodamoeba, E. nana* and *Trichomonas.* On the other hand, those intestinal protozoa with very resistant cyst capsules, *i. e., E. coli, Chilomastix* and *Giardia,* are less amenable to the action of this drug. These observations parallel those of various workers on the *in vivo* and *in vitro* action of emetin hydrochloride and yatren (iodoxyquinolin sulphonic acid) on these several intestinal protozoa.

The results obtained from this preliminary series of cases indicate the valuable place which di-hydranol may attain as an amebicidal therapeutic, particularly in view of the ease with which it may be administered and the essentially negligible toxic effects which it produces. It appears to be a particularly satisfactory therapeutic for chronic and carrier cases in children as well as in adults, and for those patients where hospitalization is impracticable.

5034

Experimental Acute Amebic Colitis in Dogs.

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Cases of spontaneous dysentery in dogs, in which an endamoeba similar in form and habits to *E. histolytica* was abundant in the exudate, have been reported by Darling,[1] Ware,[2] Fischer[3] and Bauche and Motais.[4] Three of these reports were on single infections; Ware's series comprised 7 dogs. On at least 5 different occasions the dog has been utilized experimentally to study strains of the dysentery ameba from human sources. The first of these was Lösch,[5] who was successful in one of 4 attempts in injecting into his experimental animals the cellular exudate from his classical case of the infection. This series, as well as the later cases (Kruse and Pasquale,[6] Harris,[7] Kartulis,[8] and Dale and Dobell,[9]) indicate that infection was successful only from rectal injection, that it was believed to be dependent in part on the age of the dog, and that some infections resulted in death, while others cleared up spontaneously. On the whole, the unsuccessful attempts to employ dogs for a study of human strains of *E. histolytica* have resulted in more recent years in utilizing the kitten and rhesus monkey for this purpose.

The present study was the result of the discovery of amebic dysentery in 2 dogs picked up on the streets of New Orleans. One of the animals died before the material could be utilized, but the other was estimated to have been first observed one or 2 days after the onset of the dysentery. From this animal the strain has been transferred through 6 successive sub-plants, in which 14 dogs have been utilized. Thirteen of these dogs have become positive for the infection. Only enough dogs have been used to provide for the continuance of the strain and to study the effect of the organism on the host. The ameba has also been successfully implanted into one of 2 rhesus

[1] Darling, S. T., *Proc. Med. Assoc. Isth. Canal Zone*, 1915, vi, i, 60.
[2] Ware, F., *J. Comp. Path. and Therap.*, 1916, xxix, 126.
[3] Fischer, Walther, *China Med. J.*, 1918, xxxii, 13.
[4] Bauche, J., and Motais, F., *Bull. Soc. Path. Exot.*, 1920, xiii, 161.
[5] Lösch, F., *Arch. Path. Anat.*, 1875, lxv, 196.
[6] Kruse, W., and Pasquale, A., *Z. Hyg. u. Infekt.*, 1894, xvi, 1.
[7] Harris, H. F., *Arch. Path. Anat.*, 1901, clxvi, 67.
[8] Kartulis, S., *Handb. d. path. Mikroorg.*, 1913, 2nd edition, vii, 651.
[9] Dale, H. H., and Dobell, C., *J. Phar. and Exp. Therap.*, 1917, x, 399.

monkeys, although attempts to infect 4 laboratory rats and 2 cats were unsuccessful.

The technic employed has consisted in the transfer of 1 to 2 cc. of bloody mucus exudate containing the active amebae from the cecum of an acute case of the disease through the large bowel into the distal 5 cc. of the ileum of the animal to be infected. This has been made possible by utilizing a pipette of 30 cm. length and 7 mm. outer diameter to which a 25 cc. rubber bulb is attached. The tube is gently inserted into the rectum of the first animal and with a careful pressure is advanced through the colon and cecum until the ileo-cecal valve is encountered. The bulb is then compressed and the air which is expelled is usually sufficient to cause the relaxation of the ileo-cecal valve. The tube is then slowly withdrawn as the pressure on the bulb is gradually released, thus allowing the exudate from the cecum and upper levels of the colon to be aspirated into the pipette. The material without leaving the pipette is then inserted up the large bowel and injected into the ileum of the second dog, which has been prepared by having all solid fecal matter removed by enema from the large bowel. In this way the amebae are placed in a location from which they will enter the large bowel in a similar manner to that in which natural amebic colitis is acquired. The same technic is employed on each successive day to determine the conditions under which the infection actually comes to involve the tissues.

The incubation period. Dale and Dobell (l. c.) found that the incubation period of human strains of *E. histolytica* in kittens was 1 to 6 days, with an average of 2 days. In this series of dogs one animal became infected in 24 hours, 6 in 2 days, 2 in 3 days, one in 4 days, one in 5 days and in one dog (an old animal) the infection was not observed until the twenty-third day. Infection in this series was defined as the recovery of abundant mucus with or without red blood cells, but in which, on microscopic examination, motile *E. histolytica* were readily found.

The age of the host. Dogs from 2 months of age up to 4 years have been employed. The only animal in which infection has not been developed was a 3-months-old puppy.

Course of the infection. The onset has usually been sudden, with the copious evacuation of blood and mucus a day or two after the animal was first observed to be "infected". In most animals the infection has been acute, the host dying in 2 to 3 weeks after inoculation. In one case the condition, now of several months' standing, has become chronic; in another, the 4-year-old dog, spontaneous

recovery has apparently developed. In 2 members of the series with acute amebic colitis the animals have been completely relieved of symptoms and the freshly withdrawn bowel contents have become microscopically negative after administration of di-hydranol.

The amebae and their relationship to the tissues. In no case have cysts of the amebae been recovered from the bowel evacuations, but motile *E. histolytica* have frequently been observed in freshly passed exudate. In the earliest stage following injection the amebae occur free in the mucus of the cecum or loosely adherent to the cecal epithelium, without ingested red blood cells. Twenty-four to 48 hours later the presence of ingested red blood cells and the abundance of host tissue elements from the cecal contents indicates that the organisms have probably invaded the tissues. Their recovery as tissue parasites from the colon and rectum occurs from one to several days later. These amebae are easily cultivated *in vitro* in a modified egg-Ringer's medium. Autopsy records show the largest number of lesions with their accompanying amebae in the cecum. Lesser foci have been found in order of frequency in the several levels of the colon, the rectum, the appendix and the distal 10 cm. of the ileum. This corresponds for the most part to the infection in man.

On the whole, however, the lesions in the dog are less discrete and more superficial in character than in the human host. In some animals the leukocyte count apparently indicates a bacteremia, the nature of which has not yet been determined. It is not improbable that in acute infections with fatal termination the bacterial invaders may actually play the primary rôle in producing death.

The significance of natural amebiasis in dogs. All the evidence in the literature, as well as that in this series of dogs preponderates in favor of the belief that the ameba in canine amebic colitis is the same organism as *Endamoeba histolytica* of man. Since cysts are apparently not formed in any type of the infection in dogs, the dog is not likely to be a menace to man or to other dogs. The inference is, therefore, that the dog receives its infection from man.

Summary and Conclusions. 1. By utilizing a technic whereby muco-sanguinous exudate from active cases of amebic colitis in dogs is injected *per anum* into the distal portion of the ileum, infection corresponding to spontaneous amebiasis in this host has been produced in 13 out of 14 dogs (93%), ranging in age from 2 months to 4 years. 2. The incubation period, as determined by the presence of active *Endamoeba histolytica* in mucus withdrawn directly from the cecum, has ranged from one to 23 days, with the largest number of findings on the second day. 3. Acute cases, prob-

ably complicated by bacteremia, terminate with death in about 2 weeks. Chronic cases and one spontaneous recovery have also been observed. Carrier cases, with the passing of cysts in the stools, apparently do not develop in dogs. 4. The cecum is the primary seat of infection with *E. histolytica* in the dog. 5. The dog apparently receives its amebic infection from man but due to the failure to produce cysts the infection apparently cannot be transferred in nature from dog to dog or from dog to man.

5035

Observations on the Significance of the Agglutinins and Lysins Produced by Typhoid Inoculation.

ROY F. FEEMSTER. (Introduced by C. W. Duval.)

From the Department of Pathology and Bacteriology of Tulane University of Louisiana.

In a previous paper the results of agglutination reactions performed upon the sera of students inoculated with typhoid vaccine were reported. Two sets of tests were carried out, one with serum taken before inoculation, the other with serum taken 10 days after inoculation.

This paper deals with a third set of agglutination reactions, together with parallel complement fixation reactions and bacteriolytic tests, performed upon sera from the same group of 90 students 4 months after inoculation. The antigen used in the complement fixation reactions was a saline suspension of typhoid bacilli from which lipoids had been extracted with alcohol and ether. The bacteriolytic tests were carried out by diluting one capillary drop (about 0.025 cc.) of serum to 0.5 cc. with saline, and adding to this about 500 to 1000 typhoid bacilli. This mixture was incubated for one hour and then plated in plain agar. In negative control sera there was practically always a marked multiplication of the bacilli.

Because of the marked difference in the agglutinin titer between students who had received previous typhoid vaccine and those who had not, a second inoculation was given to a number of volunteers from the latter group. Each of the tests mentioned above were performed upon sera drawn from these students 10 days after inoculation.

Analysis of the data collected in this study reveals the following observations: 1. Agglutinins are demonstrable more universally 4

months after inoculation in sera of those who have had typhoid (100%), or previous typhoid vaccine (81%), than in sera of those who have had neither (47%).

2. Agglutinins are maintained in higher titer for 4 months by those who have had typhoid (1/50), or previous vaccine (1/54), than by those who have had neither (1/28).

3. The percent of students showing agglutinins in titer strong enough for diagnosis (Widal Reaction, 1/40 dilution) 4 months after inoculation was higher among those who have had typhoid (75%), or previous typhoid vaccine (60%), than among those who have had neither (18%).

4. The percent of students showing positive complement fixation tests was slightly higher among those who have had typhoid (75%), and previous typhoid vaccine (75%), than among those who have had neither (65%).

5. The average complement fixing titer was higher among those who have had typhoid (1.6—almost++), than among those who have had previous typhoid vaccine (1.2), or neither (1.0).

6. The percent of students showing marked bacteriolytic power (over 90% of typhoid bacilli killed) was higher among those who have had typhoid (63%), and previous typhoid vaccine (50%), than among those who have had neither (31%).

7. The average bacteriolytic titer was somewhat higher among those who have had typhoid (87%), than among those who have had previous typhoid vaccine (77%), or neither (77%).

8. There is some correlation between the strength of the agglutination and complement fixation reactions. (Correlation coefficient $+0.212 \pm 0.067$.)

9. There is practically no correlation between the complement fixing antibodies and the bacteriolysins produced. (Correlation coefficient +0.068.)

10. There is practically no correlation between the agglutinins and bacteriolysins produced. (Correlation coefficient +0.099.)

11. The immunity resulting from a second inoculation some months after the first is much greater than that produced by the original inoculation, as shown by the agglutination reactions of 12 students given a second inoculation. (Before first inoculation no agglutinins, 10 days after 1/69, 4 months after 1/36; 10 days after second inoculation 1/322.) There was a similar exaltation in the complement fixation and bacteriolytic reactions.

Conclusions. 1. The immunity produced by a single course of 3 doses of typhoid vaccine may not be as great as usually believed.

A second inoculation probably ought to be given some weeks or months after the first. 2. The Widal Reaction is much less reliable as a test for typhoid fever among those who have had more than one inoculation with vaccine than among those who have had only a single inoculation.

5036

Effects of Sodium Citrate on Bile Salt Hemolysis.

JOHN W. WILLIAMS. (Introduced by W. H. Harris.)

From the Department of Pathology, College of Medicine, Tulane University.

In a previous report[1] we observed an increase in toxicity of bile salts* injected intraperitoneally into the white mouse when sodium citrate was added. In the present study the effects of varying dilutions of bile salts and sodium citrate on human blood cells *in vitro* have been observed.

When bile salts are added to red blood cells of different individuals and allowed to stand at room temperature for 18 hours, hemolysis occurs through dilutions of 1:200 to 1:6400. The bloods of 100 undiagnosed individuals were examined in order to determine their susceptibility to bile salt hemolysis. The large variation in ability of bile salts to hemolyse different bloods is independent of changes due to age of cells, and independent of the temperature and humidity under which the cells are kept.

When sodium citrate is added to bile salts, hemolysis of red cells takes place in higher dilutions of the bile salts than when the bile salts *per se* are employed and the dilution at which hemolysis takes place depends upon the concentration of the sodium citrate added. If concentrations of less than 0.032% (1:3200) of sodium citrate are added there is no increase in the hemolytic power of bile salts; however, as the concentration of sodium citrate added is increased above 0.032% the hemolytic power of the bile salts is likewise increased. For example, if 1% (1:100) sodium citrate is added to the bile salts dilutions, hemolysis takes place in the 1:400 dilution, while if 4% (1:25) sodium citrate is added hemolysis takes place in the 1:1200 dilution of bile salts. We see by these figures the gradual increase in hemolytic power of bile salts produced by addi-

[1] Williams, J. W., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 637.

* Merck & Co., about 47% sodium taurocholate.

tion of sodium citrate from the 0.032 to 1% concentrations and the rapid increase from the 1 to 4% concentrations. This increase in hemolytic power of bile salts rises further as concentrations of sodium citrate greater than 4% are added. The above is an example of the hemolytic reaction of one individual. It is typical of the reactions of all individuals with the exception that susceptibility of different bloods to bile salt hemolysis varies and the reactions would vary in accordance. Sodium chloride also increases the hemolytic power of bile salts but to a less marked degree. In all cases normal saline was used as a vehicle and dilutant since with this solution the controls of citrate, saline and glucose showed no hemolysis, whereas hemolysis did occur in the higher dilutions when distilled water was employed as a solvent. Beef serum was also used as a vehicle and dilutant of bile salts and sodium citrate which were titrated against beef red cells suspended in beef serum. In the later titrations with the serum vehicle, sodium citrate increased the hemolytic power of bile salts but to a considerably less marked degree than when normal saline was used as vehicle and dilutant for the beef cells, sodium citrate and bile salts. It can be said that bloods of different individuals show a variation in susceptibility to bile salt hemolysis.

Sodium citrate and to a lesser degree, sodium chloride in proportion to their concentrations increase the extent of the dilution of bile salts in which hemolysis of red cells takes place while glucose manifests no such effect. When beef serum is used as a dilutant and vehicle in place of normal saline, the hemolytic activity manifested on beef cells by dilutions of bile salts with different concentrations of sodium citrate added is similar but less active than that manifested when normal saline is used as a vehicle and dilutant. This protective mechanism afforded the body by its serum against hemolytic agents has long been recognized.

The varying susceptibility of different bloods to hemolysis by bile salts *per se* and the increased hemolytic effect manifested on adding sodium citrate to bile salts offers a means of determining red cell fragility. With known concentrations of bile salts and sodium citrate and with red cells of known fragility, it should be possible to determine the amount of bile salts in an unknown serum. Since under certain conditions the bile salt content of the blood varies, a means of perfecting the above determinations should serve as an index to the potential danger in blood transfusions of reaction from citrated blood due to the high bile salt content of the blood of the donor or recipient or both.

5037

Changes Produced in Certain Constituents of the Blood by Dilutions of Sodium Citrate.

JOHN W. WILLIAMS. (Introduced by W. H. Harris.)

From the Department of Pathology, College of Medicine, Tulane University.

While working upon the relationship of sodium citrate to bile salt hemolysis, interest was aroused in the microscopic picture produced in blood by addition of this sodium salt. As far as has been ascertained the changes observed have not been heretofore described, nor has it been explained in what manner a 30% sodium citrate solution stops hemorrhage when injected intravenously or intramuscularly, whereas the less concentrated solutions (4-10%) act as anti-coagulants when added to blood for transfusion.

In our observations fresh defibrinated blood, serum from clotted blood, citrated blood and blood added to the dilutant from the finger tips were studied. In each case the findings corresponded. The solutions of sodium citrate used varied in dilutions from 0.2 to 50% by weight and in all cases distilled water was used as vehicle. Saline (0.2 to 30%) was run in comparison but findings with this salt were limited to crenation in the hyper and swelling with chromolysis in the hypotonic dilutions.

When blood serum is added to a 20-50% solution of sodium citrate a flocculent, milky precipitate is formed. This redissolves macroscopically on adding more serum. This precipitate also forms in 20-50% sodium citrate solution when serum which has been passed through a Berkefeld filter or has been kept at 55°C. for an hour is added. Positive tests for globulin are obtained from the precipitate, which microscopically appears as a coarse granular material with a tendency to aid in the clumping and enmeshing of red and white blood cells and which when observed under the microscope does not disappear *in toto* on the addition of more serum. Fragmenting leucocytes are seen in 20-50% dilutions of sodium citrate with thread-like filaments projecting from apparently injured portions of their surface membrane. These filaments appear to join the granular meshwork of the serum produced by these concentrations of the sodium citrate. In the 20-50% dilutions of the salt it is noted that the red cells are flat, irregular and crumpled (more marked in the higher concentrations) and appear in shape like paper plates, first knotted up and then let resume their original form as best they can. This flattening appears to aid in the clumping (30%

dilution especially) of the cells which clumping is irregular in outline. As the concentrations of sodium citrate decrease the cells tend to resume their normal size. In the 10% sodium citrate concentration there is considerable difference in size of the reds, less irregularity in their shape, and many of them appear to have a narrow outer ring of hemoglobin and a clear center. In the 5% solution of sodium citrate the red cells are smaller and the centers appear as clear spaces shaped like crosses, stars and round discs, while in the 1% dilution these clear centers become much smaller and disappear in the next larger dilutions. In the 1 and 5% sodium citrate dilutions white cells are sometimes seen 2 to 5 times their normal size with nuclei which have lost their polymorphous character (in the case of the polymorphonuclear leucocyte) and appear as grayish blue balls set in a clear outer ring (ring and nucleus occupy each about ½ the diameter of cell) containing many small red granules, some of which loosen themselves from the cell and float off in the solution. The outline of these white cells is slightly jagged and we have designated them asters because of their resemblance to the flower of that name. Another type of altered white cell occurs in like concentrations resembling in appearance a cross section of a pearl with a darker round nucleus set into a non-transparent, non-granular ring. These latter cells are regular in outline and many of them are larger than normal leucocytes. In the 0.5 and 0.2% dilutions swelling of the red cells, especially marked in the 0.2% dilution of sodium citrate, with resultant chromolysis and shadow (ghost) cell formation is seen.

In our studies we have noted 2 forms of crenated cells and feel they should be differentiated. The flat, misshapen paper plate type seen in the 20-50% sodium citrate dilutions and the smaller hand grenade shaped type with projecting knobs, present in all blood which has stood a short time, have nothing in common either as regards outer appearance or physico-chemical forces which cause their formation. The hand grenade shaped type we have designated as cockleburrs because of their resemblance to the seed of that name. In the latter are small conglomerate granules of hemoglobin often adjacent to or in the knobs, which granules on lowering the tonicity of the solution, detach themselves and leave empty teats projecting. The cockleburr form was present in all dilutions of sodium citrate examined, with the exception of the 0.2% dilution in which remnants of the cockleburr in the form of swollen, round, colorless cells with projecting teats were seen.

In an attempt to ascertain whether the granular material which

precipitated on the addition of serum to 20-50% sodium citrate would form when serum was not present, blood cells were washed with normal saline (20-50% sodium citrate dissolved in normal saline on the addition of serum produces this precipitate) and added to 20-50% dilutions of sodium citrate, but there was no precipitate or granular deposits and in addition a less marked clumping of the cells and a lesser flattening and irregularity of the red cells. When serum is allowed to stand a considerable time over a clot, less precipitate forms on addition to 20-50% sodium citrate and there is a slight variation in the amount of precipitate with the serum of different individuals. There was no typical rouleux formation as seen in normal blood in any of the dilutions (50, 40, 30, 20, 10, 5, 1, 0.5, and 0.2%) of sodium citrate to which red cells had been added.

In summary, it may be said that we believe there is a change produced in the serum, red and white cells and platelets by the 20-50% dilutions of sodium citrate which change is not entirely reversible on the addition of more serum and the resultant macroscopic clearing of the solution which takes place. The above changes would be more apparent in the muscle tissues after intramuscular injection than in the blood after intravenous injection because of remote possibility of great dilution in the former case. We believe the changes effected by 20-50% sodium citrate and described herein, namely, globulin precipitation and change in the cellular elements of the blood, fix the sodium citrate and stimulate the activity of coagulation on the part of the body. It is further thought that the calcium is not fixed (as in the case of lesser concentration of sodium citrate) in the 20-50% concentrations of sodium citrate; it is therefore available to aid in coagulation. Temporary decrease in coagulability of blood in intramuscular injections of 30% sodium citrate might be explained by the fact that there is an early small absorption of less concentrated sodium citrate into the blood stream and a resultant temporary fixation of the calcium in small part before reactions promoting increase in coagulability are effective.

5038

A Characteristic Exanthem in Epizootic Plague and the Experimental Reproduction of This Lesion in the Guinea Pig.

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Just subsequent to the outbreak of the epidemic of bubonic plague in New Orleans, the occurrence of a concurrent rodent plague was revealed. Every rat trapped, captured or found dead, either from disease or from fumigation, was sent to the Health Department Laboratory for full investigation.

Because of the occurrence of this epizootic, the opportunity for study of approximately 150,000 rats was presented. While engaged in this routine work, we observed that many of the rats presented a peculiar discoloration of the feet. This discoloration occurred in the living trapped rats, as well as those that were dead. The lesion was so striking in appearance that the investigation of its significance seemed justified. A special study of the rats having this discoloration was made and it was noted that, in all instances, these animals were proven to be infected with plague.

Character of Lesion: The color presented is very apparent in the feet of the infected rodent animal and occurs in both extremities; it varies from a pink to a salmon tint. The lesion is clear cut, having a distinct line of demarcation and varies in its extent of distribution. In slight infections, it is confined to the feet alone, covering both the dorsal and plantar aspects, hence the appellation "pink feet" was applied. In more marked infections, the color extends over the entire extremities and, at times, appears upon the abdominal and thoracic walls. The discoloration is not due to petechial hemorrhages such as seen in hog cholera or the hemorrhagic septicemias nor is it analogous to a scarlatina erythema, but rather suggests the tissue discolorations that are seen in carbon monoxide poisoning. It appears that there occurs either some hemolytic change, causing a laked blood aspect or that the hemoglobin is, in some manner, modified and permeates the structure. The reason for the localization of this color has not been ascertained.

The occurrence of this peculiar marking was found in the greater majority of rats in which plague was present. Only approximately 25% of rats positive for *B. pestis* infection failed to show "pink feet." When this sign was absent, the animal was apparently only

recently infected as the lesions of the visceral organs were not so marked, although positive *B. pestis* cultures were obtained. Eventually the observations of this phenomena became so generalized in our laboratories that, before starting dissections, the workers established the custom of making a gross examination of the entire catch and sorting them into two groups, those with discolored feet and those with the normal appearing feet.

Experimental: In connection with the study of the exanthem of the epizootic plague, experiments were carried out with the view of ascertaining if this lesion could be reproduced in the laboratory animal. Two hundred and fifty guinea pigs were inoculated with materials from the rats dying of plague in which "pink feet" were present. While certain of the guinea pigs were injected subcutaneously with the heart's blood, splenic emulsion, and cultures from the heart's blood of the plague rats, the procedure, for the greater part, consisted of scarification of the abdominal parieties and inunction of the infected splenic pulp therein.

In the guinea pigs thus inoculated, all developed plague and 95% of these animals demonstrated the pedal exanthem. This lesion developed in about 72 hours after the inoculations and became intensified as the disease progressed. As in the natural rodent disease, the extent of its reproduction was dependent upon the virulence of the infection. The definite presence of plague in these guinea pigs was established through autopsy with accompanying smear preparations and the cultural recovery of *B. pestis.*

In a careful review of the literature, the type of lesion herein described and reproduced has not been heretofore reported either in rodent plague or in other infectious diseases of the rat.

Based on these observations of epizootic plague and upon the experimentally induced disease in the guinea pig, it is our opinion that the lesion described is a characteristic exanthem of rodent plague, which can be reproduced in these animals by the action of the *Bacillus pestis.*

Missouri Section.

St. Louis University School of Medicine, May 14, 1930.

5039

A Solanum From Siam in the Treatment of Diabetes Mellitus.

H. A. BULGER.

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In *Science* for December 23, 1927, Dr. Hugh M. Smith printed a letter relating the existence in Siam of solanaceous plants whose fruits appeared to have a marked effect on the sugar content of the urine of diabetes. The plant supposed to have been used was sent by Dr. A. Kerr from Siam to Kew Gardens, London, from which, in turn, it was sent to Prof. Craib of Aberdeen, Scotland, who described it as a new species, *Solanum sanitwongsei,* after the late Japanese physician, Dr. Yai S. Sanitwongsei, who first used the solanaceous fruit in treating diabetes. After strenuous efforts Dr. George T. Moore, Director of the Missouri Botanical Gardens, succeeded in getting seeds from the original plant described by Dr. Craib. Dr. Moore very kindly supplied us with ripe fruit grown at the Missouri Botanical Garden, and thus presented the opportunity to study its effect on diabetics.

The fruits resemble small tomatoes, averaging about one centimeter in diameter and become yellow or red when ripe. In Siam, patients were given 3 to 10 with each meal. When they were administered with the food of diabetics, the glycosuria was reported to clear immediately and remain absent for about 20 hours, but return unless the fruits were again taken. The diets, containing large amounts of rice, apparently were not restricted.

In this investigation, patients were given 5 to 7 ripe fruits to eat with each meal. Table I shows they had no appreciable influence on the blood sugar following breakfast in mild diabetics. There was no effect on the glycosuria. The severe diabetics represented in

TABLE I.

A study of the effect of fresh, ripe fruits of *Solanum sanitwongsei* on the blood sugar, following a standard breakfast, of mild diabetics who were on a constant dietary regime but who had not received insulin. Columns (1) record changes after the breakfast without fruits; columns (2) record changes the next day when 7 fruits were eaten with breakfast; columns (3) record changes after 7 fruits had been eaten with each meal for 2 days and with the test breakfast the third morning. Figures are mg. %.

	Case 1			Case 2		
	(1)	(2)	(3)	(1)	(2)	(3)
Fasting	205	242	259	235	228	260
1 hr. after breakfast	246	270	263	251	278	
2 hrs. after breakfast	268	264	248	231	250	296
3 hrs. after breakfast	257	250	251	220	230	277

TABLE II.

A study of the influence of *Solanum sanitwongsei* fruits on the course of severe diabetics partially controlled with insulin. Five to 7 fresh ripe fruits were eaten with each meal. The diets remained constant. There was no noteworthy effect on glycosuria.

	Case 3			Case 4			Case 5		
	Insulin before breakfast	Blood sugar 3 hours after breakfast	Total insulin for the day	Insulin before breakfast	Blood sugar 3 hours after breakfast	Total insulin for the day	Insulin before breakfast	Blood sugar 3 hours after breakfast	Total insulin for the day
	Units	Mg. %	Units	Units	Mg. %	Units	Units	Mg. %	Units
Before starting fruits	30	231	75	30	130	50	35	120	65
2nd day	30	188	85	30	137	50	35	118	65
3rd day	40	118	80	30	88	50	35	134	65
4th day	40		80	30		60	35	130	65
5th day	40	182	90	30	76	50	35	94	65
6th day	40		80	25		50	30		60
7th day	40	93	80	25	130	50	30		60
8th day							30	164	50
9th day							30		70
10th day							35	182	65

Table II had been in the hospital for less than a week and steady improvement would have been expected with the insulin and dietary regime alone. There was little evidence that the solanum fruits had any influence on their progress.

The effect of relatively large amounts of these berries on the blood sugar tolerance curve of fasting rabbits was also studied. The curves became somewhat higher, probably due to the carbohydrate contained in the fruits.

These experiences did not indicate any striking influence of this particular Solanum on the diabetic state. Fruit from other species of Solanum from Siam will be investigated.

5040

Effect of Sodium Salicylate on Intradermal Reactions of Rabbits.

O. E. HAGEBUSH AND R. A. KINSELLA.

From the St. Louis University School of Medicine.

Sodium salicylate is commonly used in the treatment of infections, especially those presumed to be due to invasion by streptococcus. The old idea that acute rheumatic fever is due to infection by streptococcus, and the recently developed conception that the disease is involved in a process of allergy to streptococcus, stimulate a study of sodium salicylate in relation to allergy to streptococcus.

In this study rabbits were inoculated with cultures of a strain of *Streptococcus hemolyticus* of low virulence. The injections were made into one of the knee joints, 0.1 cc. of broth culture being used. For intradermal tests, 0.1 cc. of filtrate from a 5-day culture in Harley's medium was used. Areas near the spine were used for inoculation. All animals were tested for native reactivity to the filtrates before being employed in the experiments. Fresh animals giving positive dermal reactions were discarded. In the use of sodium salicylate, 0.2 gm. per kilo in 5% aqueous solution were given intravenously. The injections were made slowly. Injections were made daily.

Preliminary studies showed that following the intra-articular injection of streptococci, purulent arthritis invariably resulted and persisted until the death of the animal. Blood cultures were rarely positive during the life of the animal unless an intercurrent disease such as "snuffles" intervened. When this occurred, hemolytic streptococci were found in the blood at autopsy. Ten days after arthritis was established, positive intradermal reactions were always present.

The conditions of the experiment were severe due to the nature of the infection and the death rate was high in all series. This did not seem to be considerably influenced by the use of sodium salicylate.

In the first series of animals 15 controls gave strongly positive intradermal reactions 10 days after the production of arthritis, and 8 animals, given sodium salicylate 24 hours before the production of

arthritis and at 24 hour intervals thereafter showed slight or no intradermal response. At this point the results of studies made by Griffith[1] of the reciprocally antagonistic action of benzoic acid and glycin on the growth of white rats, were considered. According to this author the deleterious effect of benzoic acid on the growth of young white rats was removed by adding glycin to the diet. The amount of glycin used was 0.47 gm. per kilo, in 4% aqueous solution. Groups of rabbits were therefore selected, of which one was composed of controls, one of animals receiving sodium salicylate intravenously, one of animals receiving glycin intravenously, and one of animals receiving sodium salicylate and glycin freshly mixed in proper solutions. In this experiment, 16 control animals gave strongly positive reactions; 28 animals receiving sodium salicylate, gave slight or no reactions; 4 animals receiving glycin alone gave strongly positive reactions, and 16 animals receiving mixtures of glycin and sodium salicylate, gave strongly positive reactions.

This effective suppression of so-called allergic reactions by sodium salicylate depends on factors as yet unknown. There is no reason to ascribe this effect to a general depressive action inasmuch as rabbits did not lose weight or appear less healthy while receiving sodium salicylate over a period of 10 days than did controls. Since the dermal reaction is not heightened by the use of glycin alone, the dermal responses obtained in animals treated with sodium salicylate and glycin combined, must be present because glycin neutralizes sodium salicylate *in vivo*. The nature of the dermal reaction itself remains obscure. Whatever the mechanism by which sodium salicylate suppresses the dermal reaction, this mechanism does not affect the nature of the vascular pathology found in these animals. The late proliferative vascular lesions were the same in the various series. When sodium salicylate was discontinued the dermal reactions tended to return, but after 20 days in 7 animals, only a slightly positive reaction was present, while after 50 days only a moderate reaction was present in 4 surviving animals. When sodium salicylate was administered to 4 animals which had well developed dermal reactions the effect was a gradual suppression of the dermal reaction. The experiments thus indicate that salicylates would be more effective in preventing the development of an allergic state following a primary infection than in removing allergy after the primary infection was fully developed.

If acute rheumatic fever is a disease in which the process of allergy is involved then derivatives of salicylic acid might conceivably

[1] Griffith, W. H., *J. Biol. Chem.*, 1929, lxxxii, 415.

be beneficial by interfering with this process of allergy. That this interference is of too little importance to modify the vascular pathology is apparent. By implication, the overlying process of allergy is unimportant and the underlying focus of infection at present reputed to be of streptococcal origin, is very important. In turn, the results of cultures of blood and joints become of great importance —a state of the question now many years old.

From these considerations it seems possible to draw the following conclusions: 1. Sodium salicylate suppresses the allergic dermal reactions of rabbits to filtrates of hemolytic streptococcus. This effect is most definite when sodium salicylate is given before the focus of infection has developed. 2. There is no relation between the presence or absence of this dermal reactivity and the character of the vascular pathology.

5041

Plasma Protein, Erythrocyte Sedimentability and Serum Lability in Blood of Normal and Tuberculous Rabbits.

L. R. JONES. (Introduced by M. S. Fleisher.)

From the Department of Bacteriology and Hygiene, St. Louis University School of Medicine.

Sedimentation of the corpuscles of heparinized blood was determined by noting the extent of fall in glass tubes with an inside diameter of 4 mm. and height of 100 mm. during an interval of 1 hour. The tubes were centrifuged to insure complete sedimentation and the sedimentation index computed as the ratio between the sedimentation observed in 1 hour and the possible maximum extent of sedimentation.

Precipitability of serum protein was determined by adding various amounts of aluminum sulphate, as contained in a unit volume of 1 cc. to 0.2 cc. of unheated blood serum in small tubes. Serum and reagent were mixed and set aside at room temperature for 1½ hours. A heavy flocculent precipitate that settled out, leaving a clear supernatant fluid, was recorded as a positive reaction.

Maximal, minimal and average values for these blood properties as observed in the arterial blood of 20 normal male rabbits, with similar values for total protein, fibrin, globulin and albumin as contained in the plasma are listed in Table I.

TABLE I. 20 Normal Male Rabbits. (Arterial Blood.)

	Max.	Min.	Ave.
Weight kgs.	3.9	2.3	2.91
Plasma—100 cc.			
Tot. Prot. gm.	8.00	5.11	6.77
Fibrin gm.	0.23	0.66	0.42
Globulin gm.	3.35	1.51	2.56
Albumin gm.	4.60	3.04	3.77
Prot. Quot.	2.7	1.0	1.51
Sedimentation Index	0.17	0.02	0.06

Incidence of minimal precipitation of serum protein with alum. sulph. %		(animals)
	0.01-0.05	0
	0.06	9
	0.07	9
	0.08	2

Departures from the limits of the normal range in these blood properties were noted upon monthly blood examinations subsequent to experimental tuberculous infection in normal and allergic rabbits. Of 5 animals inoculated (subcutem) with human-type tubercle bacilli 2 exhibited a slight and transitory increase in plasma fibrin. In none of the animals was there observed an acceleration in erythrocyte sedimentation or an increase in serum precipitability.

No change was observed in total protein or in distribution of the various plasma fractions in 11 animals inoculated (intraperitoneally) with bovine-type tubercle bacilli. Increase in erythrocyte sedimentability occurred in 3 animals. Lability of the serum was increased in 3 animals. These increases were not of great magnitude, transient and were co-existent in 1 animal.

To determine the effect on these blood properties of bovine-type infection in animals with residual foci of tuberculous infection (*i. e.*, animals in a state of allergic irritability) 5 rabbits were inoculated (subcutem) with human-type tubercle bacilli and 6 weeks later reinoculated (intraperitoneally) with bovine-type organisms. Subsequent blood examinations in this group of rabbits revealed no change in the distribution of plasma proteins and an incidence of increase in blood sedimentability and serum precipitability similar to that observed in the group of animals infected primarily with the bovine-type organisms.

5042

Experiments on Immunity of the White Rat to Cysticercus fasciolaris.

HARRY M. MILLER, JR. (Introduced by J. Bronfenbrenner.)

From the Department of Zoology, Washington University.

To date experimental evidence is lacking that animals may acquire immunity to cestode parasites as a result of periodically injected worm material or extracts of it. In fact the data concerning active immunity to any metazoan parasite are meagre and have only recently been reported.

Cysticerus fasciolaris, in the liver of the rat and mouse, is the larval stage of *Taenia taeniaeformis (crassicollis)* in the intestine of the cat. Infestation of the rat results from accidental ingestion of the onchospheres in the feces of the cat. These penetrate the intestinal wall of the rat, reach the liver via the portal system, and eventually develop into long strobilate cysticerci in connective tissue cysts of host origin. When ingested by the cat these mature in the intestine.

We have succeeded in immunizing the white rat by periodic injections of dried pulverized worm material as a 1% suspension in 0.1% HCl. Small quantities were injected intraperitoneally at 2 or 3 day intervals, and 5 weeks after the last injection both control and immunized animals were fed equal portions of a uniform suspension of onchospheres by means of a stomach tube. After a period of a month 9 control animals had from 10-42 (average 21.4) cysts, 2-5 mm. in diameter, in the liver; while for the most part the young worms in 9 immunized animals were dead, being represented by small, well-circumscribed spots (usually 1 mm. in diameter) in the liver. The average number of these was 21.1. A few control animals also showed spots (1-12) representing degenerated worms; and a few immunized animals showed, in addition to the necrotic cysts, small numbers of living cysts, usually much smaller in size than those in control animals.

The results of another experiment, in which the experimental animals were injected periodically with finely ground live worms, were equally conclusive. Some evidence was found that the acquired immunity may persist as long as 5 months. The animals of several other experiments have not yet been killed.

Some experimental evidence has also been secured that the presence of one or more huge cysts confers immunity to a superinfestation.

5043

Nuclear Changes of Nerve Cells in Acute Poliomyelitis.

W. P. COVELL. (Introduced by G. H. Bishop.)

From the Anatomical Laboratory, Washington University, St. Louis.

The pathology of acute poliomyelitis in monkeys has recently been described by Hurst[1] with special reference to changes in microglia. The object of the investigations reported here has been to supplement the above by a description of the injured nerve cells which so far in the course of the disease have escaped digestion by neuronophages. Thus certain nuclear changes in ventral horn cells and cells of the medulla have been found to follow or occur simultaneously with the more easily recognized cytoplasmic degeneration which may be profound.

Central nervous system tissues of 33 monkeys dead of poliomyelitis, or sacrificed at various stages during the course of the disease, were fixed in Zenker's fluid and stained with Giemsa for routine work. For comparison the tissues of 10 normal monkeys were available. Fixation has been supplemented by methods of Carnoy, Champy, A. O. B., Regaud, etc., and numerous staining methods have been employed including erythrosin-azur, phloxine-methylene blue, hematoxylin-eosin, phosphotungstic hematoxylin, etc. Sections for study were cut 3 to 5μ in thickness.

Areas of the cord showing lesions typical of acute poliomyelitis contain a number of faintly stained nerve cells. The cytoplasm of these cells is badly damaged and in some instances appears to be lacking. The nucleus, on the other hand, is usually severely altered but retains a nuclear membrane. It may be large and clear, presenting an appearance similar to a nuclear change described by Cowdry and Kitchen,[2] Plate V, Fig. 48, for liver cells, in cases of yellow fever. The nucleolus may be absent, broken up and plastered against the nuclear membrane, or displaced in position and ragged in shape. Usually the nucleus of these cells contains one or more acidophilic-staining bodies (Figs. 1-5), definite in their outline, and measuring from about 0.25μ to 3μ in diameter. They are surrounded by a distinct halo. The bodies can be seen in freshly isolated ventral horn cells. In fixed preparations they react negatively to the Feulgen test for thymonucleic acid, and are not doubly refractile to polarized light.

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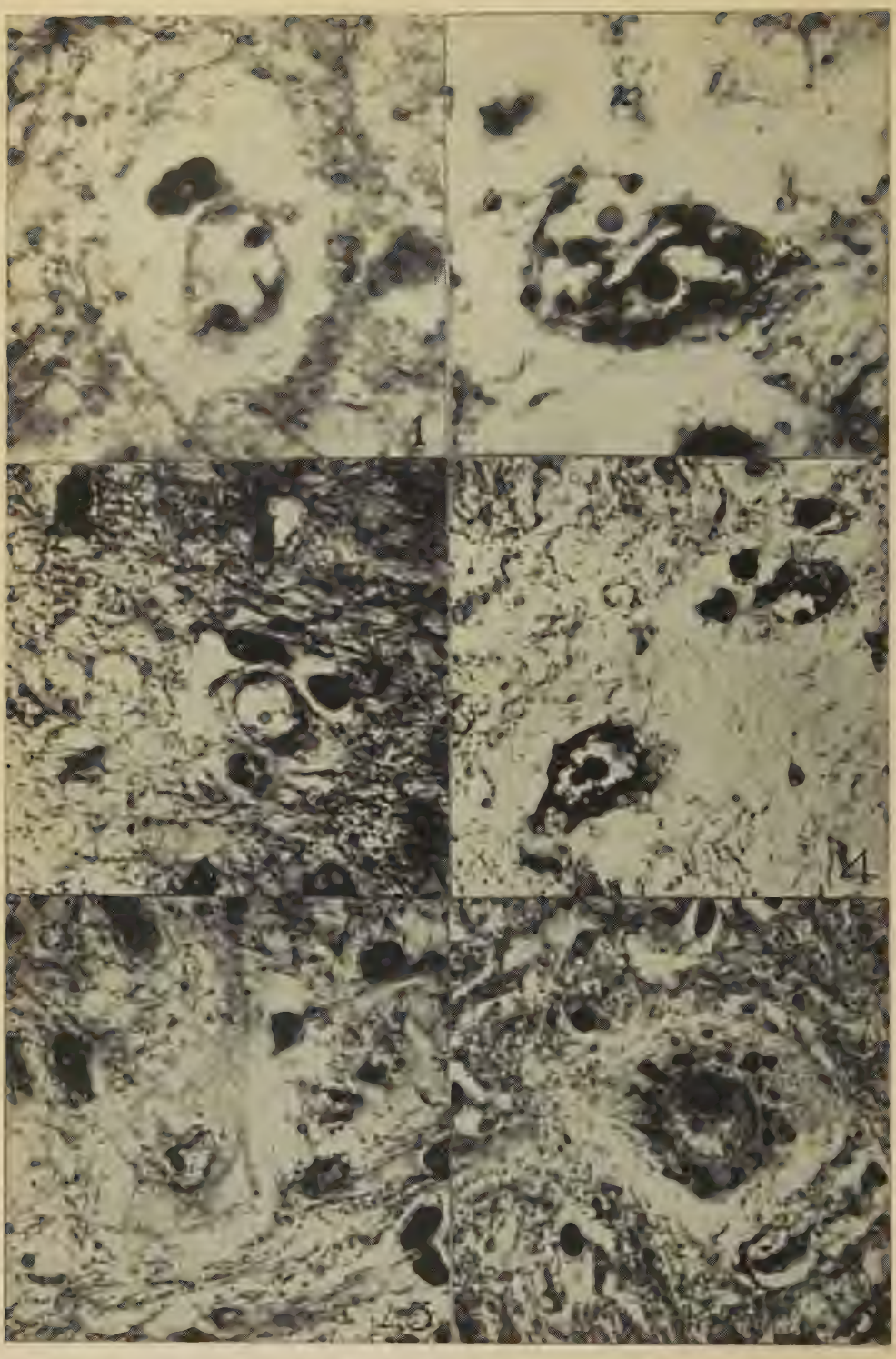
[1] Hurst, E. W., *J. Path. and Bact.*, 1929, xxxii, 457.

[2] Cowdry, E. V., and Kitchen, S. F., *Am. J. Hygiene*, 1930 xl, No. 2, 227.



The presence of these bodies in considerable numbers in cords of animals sacrificed shortly after the onset of paralysis, with fewer in animals sacrificed in later stages of paralysis (1 to 3 weeks), indicates a fluctuation in their numbers with the course of the disease. They have been observed in tissues of all monkeys sacrificed at the onset of paralysis. Whether these bodies are pathognomic of the disease remains to be determined. In any case their relation to the formation of inclusion bodies described for other filterable virus diseases might prove of value, since a splitting of the acidophilic and basophilic fractions of the nucleus is a step in the formation of specific inclusion bodies for many of these diseases. Perhaps they represent a form of oxychromatic degeneration similar to that described by Nicolau and Galloway[3] for Borna disease, and might occur in other virus diseases as well. So far, it is true that they are to be seen only in cells undergoing marked degenerative changes, but further studies may reveal earlier stages in less altered cells.

DESCRIPTION OF PLATE.

FIG. 1. Ventral horn cell from the lumbar region of the cord. Its cytoplasm is badly damaged and contains a single polymorphonuclear cell. The nucleus is clear and large with an acidophilic body at either end. Remnants of basophilic chromatin are to be seen on the nuclear membrane. X 1600.

FIG. 2. Nerve cell of the medulla containing two acidophilic bodies separated by a basophilic strand. Note the presence of the nucleolus slightly below and to the left of the upper body. X 2400.

FIG. 3. Ventral horn cell containing a single acidophilic body in a clear nucleus, with basophilic chromatin arranged on the nuclear membrane. Three neuronophages are to be seen in the cytoplasm. X 1150.

FIG. 4. Two nerve cells in the area of a lesion of the medulla, containing acidophilic bodies with basophilic chromatin in large amounts about the nuclear membrane. X 1150.

FIG. 5. Ventral horn cell with a single body at this focus. The cytoplasm is vacuolated and severely damaged. X 1150.

FIG. 6. A ventral horn cell showing the normal appearance of the cytoplasm and considerable acidophilic chromatin. X 1150.

[3] Nicolau, S., and Galloway, I. A., Medical Research Council, Special Report Series, No. 121, 1928.

5044

Some Observations on Vitamins A and D Activation of Yeast by X-Ray and Other Radiation.

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During the course of experimentation with the production of varying degrees of erythema of the skin, induced by x-ray over a small area of the body of the white rat, it was noted that animals fed a ration deficient in vitamin A and given a mild erythema dose developed symptoms of deficiency later as compared to control series.[1] It had been noted in previous experiments that a moderately large quantity of x-ray given in the form of a general body dose induced to a certain extent a vitamin A deficiency.[2] I assumed at that time that the x-ray played a rôle in the activation or utilization of vitamin A in the body and might be a source of activation of vitamin A in other forms in nature.

In the literature the greater amount of investigation has been centered upon activation of vitamin D or the antirachitic vitamin in various substances by means of the quartz light and the Cooper-Hewitt, or carbon arc lamp.

During the last two years I have studied the activation of yeast by the carbon arc, the quartz light and the x-ray. Various periods of exposure were used with each set-up. The yeast used was that of compressed Brewer's variety.* The test for vitamin A has resorted itself into a biological one in that the chemical tests with trichloracetic acid and similar chemicals as described by Fearon[3] and others, have proven to be unreliable.[4] I have used the white rat in all of these experiments and for the determination of vitamin D deficiency I have studied the bony structure by means of radiograph, silver impregnation in the case of amputated extremities, and as a check, hematoxylin and eosin stain in a few cases. The radiographic evidence will give data at varying periods of time in an animal under a period of observation.

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[1] Jorstad, L. H., and Lane, C. W., *Arch. Derm. and Syph.*, 1929, xix, 954.

[2] Burrows, M. T., Jorstad, L. H., and Ernst, E. C., *Radiol.*, 1928, vi, 7.

* The yeast was furnished by the laboratories of Anheuser-Busch, Inc. The radiation with the Cooper-Hewitt light and the quartz light was done by the same laboratory, who also granted certain amounts of money for materials used.

[3] Willimott, S. G., and Moore, T., *Biochem. J.*, 1926, xx, 651.

[4] Hawk, Phil. B., *Science*, 1929, lxix, 200.

In our studies with the Cooper-Hewitt lamp the yeast was exposed for periods from 5 to 60 minutes. There was some protectivity against rickets in the yeast exposed for 30 minutes, but complete protection was not afforded with the samples radiated less than 50 minutes. The 60 minute radiated yeast was not as protective as the 50 minutes. In regard to the vitamin A activation it was noted that xerophthalmia occurred in animals fed samples radiated up to 30 minutes and protection was not afforded completely until the radiation period was 50 minutes.

With the quartz light complete protection against xerophthalmia and rickets occurred with 5 to 7 minutes' radiation of the yeast sample. This yeast was radiated in the same manner.

In each case 5% of the yeast by weight was added to the Steenbock ration in one series and to a ration composed of egg albumin, potato starch and McCollum's salt mixture in another series. The second diet is a better one for determining vitamin A deficiency in that the cracked corn used in the Steenbock ration contains a certain quantity of vitamin A. Crisco was also added to the diet in that this supplies fat free of the fat soluble vitamin. Here again as in the experiments with the Cooper-Hewitt lamp the higher exposure, that is, 9 and 11 minutes, with the quartz light, did not give the same protection as the lower periods of radiation.

In the former experiments the amount of x-ray which had protected the rat against a vitamin A deficiency was 20 MAM, given to an area on the animal 1 cm. in diameter. Thus, in this set-up it was decided to radiate yeast cake 1 cm. thick for periods of 20, 25 and 30 MAM. The set-up used was 10 inch distance, 9 inch spark, 90 kilovolts; 4 minutes' exposure giving 20 MAM, etc. With this yeast greatest protection was afforded against rickets by that given the 20 MAM exposure.

The interesting factor in these experiments is that there is a point wherein activation reaches its maximum and then becomes less. Thus, we may speak of an activation curve with a rounded contour, the apex being reached at a different period of time with the different lights used. From that one can assume that the question involved is that of energy, the energy output of the Cooper-Hewitt lamp being less than that of the quartz and the quartz being less than the x-ray.

In former experiments we have shown that with different types of x-radiation, that is, the high voltage type with filtration, the vitamin A liberation occurs at 10 MAM and with a higher radiation, such as 75 MAM, vitamin A has to be added to the organism in

order to prevent loss of body weight during a course of several treatments. Here again we have the same energy curve as we have in the above experiments. All these experiments tend to refute the idea that certain lights are specific in the liberation of certain products. It also tends to establish that vitamin D is closely allied with vitamin A and that the question of liberation of these vitamins from yeast is a question of the differences in energy given.

Conclusions. The Cooper-Hewitt lamp, quartz light and x-ray in low voltage cause an activation of the vitamin D and the vitamin A in yeast fed to rats along with a deficient vitamin D and vitamin A ration. The period of exposure of the light in each case varies with the type of light used. The maximum point of activation is found with each light.

Western New York Section.

Cornell University, Ithaca, N. Y., May 24, 1930.

5045

Plasma Lipid Levels in Normal Post-Absorptive Dogs.

DAVID GLUSKER. (Introduced by W. R. Bloor.)

From the Department of Biochemistry, University of Rochester School of Medicine and Dentistry, Rochester, New York.

In this study 10 healthy male dogs kept under standard conditions of exercise, maintained at a weight that varied less than 5% and fed a constant diet were bled 15 hours post-absorptive over a period of a month, at intervals of 1-16 days. Analyses for total lipid were carried out on 5 cc. samples of plasma according to the methods outlined by Bloor, using also his technique for the colorimetric determination of cholesterol by the Liebermann-Burchard reaction.[1]

The reliability of the procedures claimed by Bloor was verified, the total lipid method giving a standard deviation of ±1.4% and the cholesterol ±2.6%. Significant changes in lipid levels were taken as average differences of more than twice the standard deviation, or 5.1%. It was found that the method for phospholipid (1929) was reproducible with a standard deviation of ±4.1% on stock alcohol-ether extracts and on purified petroleum ether solutions of phospholipid, but when applied to the plasma extracts from the dogs under study, low values which could not be duplicated were obtained.

The average standard deviation for 4 post-absorptive determinations of cholesterol was ±6%. The values for the individual dogs varied from 68 mg. % to 118 mg. % with a standard deviation of ±28%.

The total fatty acid levels for the individual animal varied by a standard deviation of ±7%, and the means varied from 205 mg. % to 348 mg. %, with a standard deviation of ±13%.

It is evident that the cholesterol and total fatty acid levels in an

[1] Bloor, W. R., *J. Biol. Chem.*, 1928, lxxvii, 53.

individual dog are relatively constant over the period of one month of controlled conditions, and that there may be large differences between one dog and another. The lowest values for cholesterol and total fatty acid were given by young dogs, and the highest values were obtained from an old dog.

If the values are arranged in order of magnitude, the sequence for total fatty acids and cholesterol are roughly the same.

5046

Accumulation of a Precursor of Lactic Acid in Muscle After Epinephrine Injections.

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From the State Institute for the Study of Malignant Disease, Buffalo.

It has been found recently[1] that the glycogen content of the rat gastrocnemius diminishes on an average by 101 mg. % 30 minutes after an epinephrine injection, while the lactic acid content of the muscle increases by only 29 mg. %. Part of the lactic acid formed in muscle diffuses into the blood but even if one allows for this on the basis of the increase in blood lactic acid (which amounted to 21 mg. %), one arrives at a total of only 40 mg. % lactic acid. Hence, 61 mg. % of the muscle glycogen which disappears remains to be accounted for.

Three hours after an epinephrine injection, when the blood lactic acid has returned to the original level, a much larger portion of the disappearing muscle glycogen can be accounted for.[2] The liver glycogen formed from blood lactic acid including that part which is mobilized again, makes up for 83% of the glycogen lost from the muscles. The rest is such a small amount that it might have been oxidized without appreciably affecting the R.Q. which was 0.715 for the 3 hour period after the injection. Since most of the muscle glycogen which remains unaccounted for 30 minutes after the injection eventually (within 3 hours) yields liver glycogen by way of blood lactic acid, it seemed desirable to look for a substance, intermediary between glycogen and lactic acid, which might have accumulated in muscle owing to the fact that the conversion of glycogen

[1] *Am. J. Physiol.*, in press.

[2] Cori, C. F., and Cori, G. T., *J. Biol. Chem.*, 1928, lxxix, 309.

into the precursor proceeds more rapidly than the conversion of the latter into lactic acid.

It seems fairly well established that the lactic acid precursor in muscle is a hexose-phosphoric acid ester. A fall in the inorganic phosphates of the blood and in phosphate excretion in the urine has been observed after epinephrine injections[3] but the nature of this decrease has not been explained. In the experiments referred to above[1] a decrease in the inorganic phosphates of muscle amounting on an average to 5.8 mg. % has been found 20 minutes after the epinephrine injection, while there was no change in phosphocreatine. If one assumes the formation of a hexose-monophosphoric acid, this would account for the disappearance of 24 mg. of glycogen.

The methods for the determination of hexose phosphoric acid in tissues have not been worked out completely and in view of the difficulties experienced in the past great caution should be exercised. Embden and Jost[4] made use of the fact that hexose phosphoric acid is precipitated by ammoniacal alcohol in the presence of an excess of magnesium ions. Their method is being subjected to a thorough examination and this will be reported later. Since the magnesium precipitate includes varying amounts of other phosphorus compounds only the hexose can be determined with any degree of certainty at the present time.

Rats, starved previously for 24 hours, were anesthetized with amytal and one gastrocnemius muscle was removed by cutting it at the tendons. The second muscle was extirpated one half hour later. The muscle was immediately thrust into a tared tube containing ice-cold trichloracetic acid, was thoroughly ground with sand and the filtered extract was allowed to stand until the next day. The precipitation of the hexose phosphoric acid was carried out as described by Embden and Jost and the reducing power was determined by means of the Hagedorn and Jensen method. The following values given in mg. of glucose per 100 gm. muscle were obtained in 6 control animals.

Muscle I—61.0; 55.3; 41.2; 41.7; 47.5; 53.2; ave. 50.0.

Muscle II—64.8; 55.8; 38.8; 43.0; 48.6; 52.3; ave. 50.5.

When 0.02 mg. of epinephrine per 100 gm. rat was injected subcutaneously immediately after removal of muscle I and muscle II extirpated 30 minutes later, the following results were obtained:

Muscle I—42.3; 55.3; 45.6; 39.2; 46.2; 45.5; 48.8; ave. 46.1.

[3] Perlzweig, D. A., Latham, E., and Keefer, C. S., PROC. SOC. EXP. BIOL. AND MED., 1923, xxi, 33.

[4] Embden, G., and Jost, H., *Z. Physiol. Chem.*, 1928, clxxix, 24.

Muscle II—65.5; 94.0; 59.0; 86.5; 85.7; 71.7; 88.3; ave. 78.7.

Lactic acid (40 mg.) and its precursor (32 mg.) are thus seen to account for 73% of the muscle glycogen which disappears in 30 minutes after the epinephrine injection. Since a deposition of liver glycogen from blood lactic acid occurs shortly after the injection, part of the remaining 28 mg. is probably accounted for by this process.

A decrease in the inorganic phosphates of the blood has also been observed after insulin injections.[5] Since muscle glycogen increases under these conditions, it seems possible that the same chemical transformations occur as after epinephrine injections but in the opposite direction. This is being investigated at the present time.

5047

Cinematography of the Vocal Cords.

CHARLES A. MORRISON. (Introduced by J. R. Murlin.)

From the School of Medicine and Dentistry, University of Rochester.

A new technique for making motion pictures of the vocal cords has been developed by the use of a quartz rod as the means of projecting a high intensity illumination within the larynx. The source of light consists of the two filaments of an overvolted automobile bulb. A laryngoscope, illuminating system and viewing finder are attached to a 16 mm. motion picture camera. This combination forms a self-contained, one-man-controlled unit, which permits motion pictures of the cords to be made at the standard rate of 16 frames per second. The field photographed is viewed constantly through the finder by the operator, who controls the spring motor of the camera by the usual release button. The pictures as projected fill the entire screen area. This is a magnification previously unattained under these conditions.

[5] Harrop, G. A., and Benedict, E. M., PROC. SOC. EXP. BIOL. AND MED., 1923, XX, 430.

5048

Effect of Exercise and the Specific Dynamic Action of Fat in Obese Subjects.

BYRON D. BOWEN AND FRED R. GRIFFITH, JR.

From the Buffalo General Hospital and the Department of Physiology, University of Buffalo School of Medicine.

The effect of a meal containing 100 gm. of butter and 50 gm. of olive oil mayonnaise, alone, and with exercise, has been determined in 2 normal and 4 non-diabetic obese subjects.

The metabolism was determined by collection and analysis of the expired air; after determining the basal metabolic rate the subjects were usually exercised while still in the post-absorptive state and before the ingestion of the fat meal; this gave the post-absorptive metabolism both at rest and in exercise. They were then given the 150 gm. of fat and the resting and exercise metabolism was determined, usually once an hour for 7 to 8 hours.

The exercise consisted of having the subject lift the lower extremities, while lying on the back and connected with the spirometer, rhythmically and alternately so as to touch with the toes a board placed a given distance above the foot of the bed. The rhythm was maintained by the use of a metronome which was usually set at about 76 beats per minute, so that a lower extremity was lifted 19 times per minute. This exercise increased the metabolism from 2 to 3 times. The subjects exercised for 2 minutes before collection of the expired air was begun; and this was continued for 5 or 6 minutes, depending on the degree of fatigue produced.

In 2 normal subjects and one of the obese there was a slight initial rise in the resting respiratory quotient following the ingestion of the fat; this was followed by a gradual fall, which, at the end of 6 to 7 hours reached a level lower than the resting quotient.

In 3 of the 4 obese subjects there was a rise in the resting quotient following the fat meal. In one of these, who was observed on 3 different days, the quotient reached unity twice and 0.94 the remaining time; in the former it was still one at the end of 5 hours. In another subject the quotient rose from 0.73 to 0.83 at the end of 3 hours and a quarter and was still 0.78 at the end of 6 hours and a quarter. In the remaining subject the rise was from 0.74 to 0.93 at the end of 6½ hours.

The respiratory quotient during the periods of exercise after the fat meal was always lower than that of the resting metabolism in all

of the subjects studied. This reduction was considerably more in the obese subjects, in whom the resting quotients went highest, than in the normals.

As the butter was given in 150 cc. of clear, hot broth; and the mayonnaise with 50 gm. of lettuce, the effect of the hot broth and lettuce alone was observed in 1 of the obese subjects. Under these conditions the quotient fell from 0.84 to 0.71 but during exercise rose to 0.88. There was no specific dynamic action.

In general we found that when the respiratory quotient was high, exercise caused it to fall and when it was low, exercise caused it to rise. The fall at high quotients, however, was greater than the rise at low quotients. In this respect these results are similar to those found by Krogh and Lindhard,[1] whose subjects, however, were studied in the post-absorptive state.

The CO_2 capacity of the plasma in many of the subjects was determined in the basal state and after the fat meal before the rest periods. No significant change was found. Also we found that the exercise did not affect the CO_2 capacity in one of the obese subjects studied.

There was no essential difference in the maximum specific dynamic action in the normal individuals and in the obese. The average was about 13%.

5049

The Hormone of the Adrenal Cortex.

FRANK A. HARTMAN AND KATHERINE A. BROWNELL.

From the Department of Physiology, University of Buffalo.

We have previously[1] demonstrated that an extract which will definitely prolong the lives and ameliorate the symptoms of adrenalectomized cats can be made from the adrenal cortex.

We have proposed the name of cortin[2] for this hormone, which is essential to life.

Heat (80°C. for 5 minutes) destroys it. It is lost upon repeated

[1] Krogh, A., and Lindhard, J., *Biochem. J.*, 1920, xiv, 290.

[1] Hartman, F. A., MacArthur, C. G., and Hartman, W. E., PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, 69.

[2] Hartman, F. A., Brownell, K. A., Hartman, W. E., Dean, G. A., and MacArthur, C. G., *Am. J. Physiol.*, 1928, lxxxvi, 353.

precipitation with NaCl. Therefore some other method of concentration must be employed.

An extract of any desired concentration can be obtained by extracting the cortex with ethyl ether. After removing the ether *in vacuo* the residue is extracted with warm 80% alcohol. Chilling precipitates much inactive material. Removal of the alcohol *in vacuo* is followed by extraction of the residue with water to make the desired concentration, or extraction by alcohol is repeated for further purification.

Completely adrenalectomized cats treated with this extract not only live indefinitely in good condition but are able to meet unusual demands apparently as well as normal animals. They undergo major operations. Wounds heal promptly. They seem to resist infections to which untreated adrenalectomized cats often succumb. One cat had an abortion following the removal of the second adrenal and bled for several days afterward. Yet, by the use of this extract she recovered. Another cat was etherized and thoroughly explored for accessory adrenals 136 days after the removal of the second adrenal, with recovery as prompt as would be expected in a normal cat.

If adrenalectomized cats are given more extract than is necessary to keep them in fair condition they eat more and gain in weight. Blood urea remains within the normal range.

One adrenalectomized cat has been rescued from the final stage of prostration due to an inadequate supply of cortin 3 times by injection of extract. The last time dyspnea and convulsive twitchings had developed. Seventy minutes after the injection of the extract the cat was sitting up. In 85 minutes she was shivering. In 100 minutes she was eating, not merely tasting but taking her usual quantity of food.

Individual animals show great differences in the amount of cortin which they require as well as the frequency of injection needed.

5050

Relationship Between the Reaction and Phosphate Concentration of Urine Specimens Collected Over Short Periods.

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From the Laboratories of the Clifton Springs Sanitarium and Clinic, Clifton Springs, New York.

Charts and tables showing relationships between the urinary reaction and phosphate concentration in short period experiments on subjects taking normal diets and eating a standard meal were prepared. The experimental technique has been described elsewhere.[1] The Bell-Doisy method was used in determining the phosphate concentration.[2] When equal reactions were found on 2 specimens of urine obtained at about the same time the actual pH values were usually fairly low, but there was a second, smaller group in which they were more alkaline. The reactions of relatively few specimens fell between pH values of 6.0 and 7.0. The authors believe that the former, larger group, probably represents a true physiological constancy of the reaction, while the smaller one where the reaction is more alkaline they think frequently contains instances in which those changes in the reaction of urine which take place after a specimen has been voided[3] have taken place under approximately constant conditions, and have produced a constancy of reaction which may have little physiological significance. The phosphate concentration was much higher in the more acid than in the more alkaline group.

Further study of the material brought out the following points: 1, when the reactions of 2 specimens of urine obtained from the same patient differed by 0.2 pH or less the average of the means of the phosphate concentration in the urine was about 20% higher than in the whole series; 2, when the reactions of 2 specimens differed by more than 2.0 pH the average of the means was 50% lower than in the series considered as a whole; 3, when the mean phosphate concentration was high very few specimens showed differences in reaction which were greater than 0.2 pH; 4, when the mean phosphate concentration was low about half of the specimens, even from patients with achlorhydria, showed differences in reaction greater than 0.2 pH; 5, the average mean pH was lower when the

[1] Munford, S. A., and Hubbard, R. S., *J. Am. Med. Assn.*, 1926, lxxxvii, 922.

[2] Bell, R. D., and Doisy, E. A., *J. Biol. Chem.*, 1920, xliv, 55.

[3] Marshall, E. K., Jr., *J. Biol. Chem.*, 1922, li, 3.

phosphate concentration was low than when it was high; 6, in many individual experiments there was little or no correlation between changes in reaction and in phosphate concentration; 7, the average phosphate concentration was higher in achlorhydria, possibly due to the small hourly volume of urine excreted, than it was in a short control series.

It seems probable, therefore, that the phosphate concentration produced in the course of ordinary metabolism modifies the urinary reaction as do the variations produced by various experimental methods.[4] The relationship between phosphate concentration and the urinary reaction is not, we believe, an essential one. High concentrations of phosphate tend to decrease, and low ones to exaggerate variations in the urinary reaction due to various causes. Part of this buffer effect is almost certainly concerned in the changes in the reaction of the urine which take place after it is voided, but what proportion of the relationships noted should be attributed to this cause cannot be decided. It seems probable that variations in the phosphate concentration may explain some of the unusual findings noted in our studies upon the alkaline tide in urine; it seems possible, also, that the high degree of constancy of urinary reaction frequently found in patients with achlorhydria may result, not only from the relative constancy of the acid-base metabolism in these subjects, but also from the fact that the phosphate concentration in their urine tends to be high.

5051

Experimental Production of Cervical Cellulitis Resembling Ludwig's Angina.

DAVID T. SMITH. (Introduced by S. Bayne-Jones.)

From the New York State Hospital for Incipient Tuberculosis, Ray Brook, N. Y.

Infections in the floor of the mouth may extend into the deep facial planes of the neck and produce a cellulitis which has a decided tendency to terminate in gangrene or abscess formation. Ludwig originally described the condition as a gangrenous induration of the neck; Vincent refers to the disease as a foetid para-buccal abscess. The primary focus occurs about the teeth in most in-

[4] Rannenberg, E., *Arch. f. d. ges. Physiol.*, 1925, ccxii, 601.

stances (Muckleston,[1] Van Wagenen and Costello,[2] Ashhurst[3]). These observers, among others recovered an aerobic streptococcus from the cervical lesion, more rarely staphylococci or other aerobic organisms, which they regarded as presumably the causal agent. However, no reference is made to anaerobic studies. Melchoir[4] found fusiform bacilli and cocci in 4 cases; fusiform bacilli alone in one case; spirochetes, fusiform bacilli and cocci in another case. More recently, Hansen* told me he observed a fatal case of Ludwig's angina in which he found fusiform bacilli and spirochetes in large numbers.

It has been demonstrated that local lesions about the teeth generally harbor fuso-spirochetal organisms. These organisms produce a foul abscess or gangrene when introduced into the tissues of animals.[5, 6, 7] It therefore seemed reasonable to infer that Ludwig's angina might be due to a fuso-spirochetal infection.

The scrapings from the teeth of patients with pyorrhea were inoculated into the inner margin of the gum of the lower posterior teeth in 25 guinea pigs. Each of 5 pigs received 0.25 cc. of pyorrhea material. Four animals remained normal, the fifth, after 5 days, developed an enlarged gland deep in the neck immediately below the mandible. The gland gradually enlarged during the following week and the infection spread to the surrounding cellular tissue. At necropsy on the twelfth day a large amount of foul pus was found in the neck which contained innumerable spirochetes, fusiform bacilli, vibrios and anaerobic cocci. The purulent material from this animal in the amount of 0.25 cc. was inoculated into the inner margin of the gums in each of 10 normal guinea pigs. Seven of the 10 pigs developed a unilateral brawny induration in the tissues of the neck which appeared at 24 hours and rapidly grew in size and firmness until the fourth day. Five of these animals died between the sixth and tenth day, and at necropsy showed a gangrenous cellulitis which had dissected back and by pressure closed the glottis. The infection subsided by resolution in one animal and one other recovered after spontaneous rupture and drainage.

[1] Muckleston, *Ann. Otol. Rhin. and Laryn.*, 1928, xxxvii, 711.

[2] Van Wagenen and Costello, *Arch. Surg.*, 1928, lxxxvii, 684.

[3] Ashhurst, *J. Am. Med. Assn.*, 1929, xcii, 500.

[4] Melchoir, *Berl. Klin. Wochenschr.*, 1917, xxix, 695.

* Personal communication from Dr. Oscar C. E. Hansen of the Department of Medicine at the Johns Hopkins Hospital.

[5] Kline, *J. Inf. Dis.*, 1923, xxxii, 481.

[6] Pilot and Davis, *Arch. Int. Med.*, 1924, xxxiv, 313.

[7] Smith, *Am. Rev. Tb.*, 1927, xvi, 584.

The experiment was repeated with 10 other animals and 9 of these likewise developed a cervical cellulitis resembling Ludwig's angina.

Thomas[8] collected 106 cases of Ludwig's angina from the literature and found that the mortality was 40%. Van Wagenen and Costello have shown that with earlier diagnosis, earlier and more prompt surgical interference, the mortality is reduced. The administration of neo-arsphenamine or sulph-arsphenamine in the initial stages of fuso-spirochetal infections of the lung has been helpful in treatment[9] and therefore arsenical therapy would appear to be indicated in those cases of Ludwig's angina due to a fuso-spirochetal infection.

5052

Some Metabolic Changes Occurring in Prolonged Diathermy Treatments.

E. S. NASSET AND S. L. WARREN. (Introduced by J. R. Murlin.)

From the Department of Vital Economics, and the Division of Radiology of the School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.

Studies on the respiratory exchange and the sugar, non-protein nitrogen, chlorides and carbon dioxide content of blood, were made on anesthetized (morphine + amytal) dogs. Tracheotomy was done and connection made to a Benedict universal apparatus. Blood analyses were done by standard methods. The high frequency current had the following characteristics: wave length—200 meters, relatively high voltage, currents from 500 to 1000 milliamperes. Electrodes were placed on the left upper arm and right thigh, or on either side of the head. Treatment continued from 1 to 3 hours. Temperature measurements were made with thermocouples and mercury thermometers.

The respiratory metabolism invariably increased—in some cases 150%. Body temperatures were elevated 5 to 7°C. When blood sugar was initially relatively high there was a gradual depletion during diathermy; in cases of low initial concentrations a preliminary rise was noted followed by a fall. The end result was a marked hypoglycemia (30 to 50 mg. per 100 cc. blood). Non-protein nitrogen was in some cases increased to 200% of normal. Chlorides

[8] Thomas, *Ann. Surg.*, 1908, xlvii, 161.

[9] Smith, *J. Am. Med. Assn.*, 1930, xciv, 23.

failed to exhibit any gross changes. The carbon dioxide content of whole blood and of plasma invariably dropped to a rather low level (about 35 vol. %). Panting was induced in some animals, during which time the respiratory rate exceeded 250 per minute.

5053

Lipid Metabolism of Tumors.

MORIO YASUDA. (Introduced by W. R. Bloor.)

From the Department of Biochemistry, School of Medicine and Dentistry, University of Rochester.

As a preliminary report on the lipid metabolism of tumors, of which little is known, lipids of the transplanted carcinoma of the mouse as well as malignant and benign human tumors were analyzed. The total lipids and phospholipids were determined by Bloor's oxidative method, cholesterol and other unsaponifiable substance by the oxidative method with some modifications. Neutral fat was calculated by the subtraction of phospholipid and cholesterolester fatty acid from total fatty acid. The micro-oxidative determination of cholesterol of Okey was modified in that the cholesterol digitonide after saponification and washing on the glass filter was dissolved by hot absolute alcohol, the solution filtered then evaporated to dryness to get rid of alcohol and the residue oxidized. The total unsaponifiable substance was obtained as follows: The lipid solution was saponified with sodium alcoholate, then acidified and extracted with petroleum ether. The petroleum ether was then added to equal volumes of absolute alcoholic sodium alcoholate and water whereby the fatty acids are separated as soap in the alcohol-water while the unsaponifiable remains in the petroleum ether. The unsaponifiable substance in the petroleum ether is recovered by evaporation, determined by oxidation and calculated as cholesterol.

The average value of lipid analysis in tumors is tabulated: (Lipid content is expressed as gm. per 100 gm. dry tissue.)

TABLE I.

Tissue	Phospho-lipid	Cholesterol		Neutral fat	Residual unsapon. substance (% of total unsap.)
		Total	Free		
Human cancer	5.889	1.897	1.177	8.328	38.2
Mouse cancer	6.723	2.528	1.738	3.982	29.0
Human benign tumor	2.461	0.767	0.601	1.478	26.7

The high lipid content of malignant tumors is notable. The high phospholipid content in malignant tumors as compared with benign tumors seems to be of particular interest. The water content of tumors is generally high as has been reported, little difference was found in the content of residual or unknown unsaponifiable substance. The experiments are being continued.

5054

Obliteration of Vasoconstrictor Gradient in the Extremities Under Nitrous Oxide-Oxygen, Ether, and Tribromethyl Alcohol Anesthesias.

W. J. MERLE SCOTT AND J. J. MORTON.

From the Department of Surgery, the University of Rochester, School of Medicine and Dentistry.

Recent studies of peripheral vascular diseases have brought out the importance of differentiating the element of spasm from that of organic occlusion. Vasoconstriction can be demonstrated in the extremities of most individuals with normal blood vessels by measuring the surface temperatures. This action is present in varying amounts depending on the interplay of environmental conditions and the nervous mechanism. It possesses a definite gradient[1] so that it usually begins about the knee and progressively increases distally. Consequently the toes are normally the coldest parts of the lower extremity. These surface temperature differences can under certain conditions be made to disappear. In a series of 22 individuals with normal vessels it was found that this took place when the lumbar sympathetic fibres were paralyzed by spinal anesthesia. All surface temperatures of the extremities came to approximately the same level, with a variation of $\pm 1.7°$C. from the mean. This evidently represents a condition of physiological vasodilatation in the vessels of the extremities and we have called it "the normal vasodilatation level." Its importance consists in that it permits an accurate estimate of the degree of spasm in any given case of vascular disease. The failure to react to the normal level in the latter signifies the presence of organic occlusion, the degree of which is measured by subtracting the maximum temperature achieved from the

[1] Morton, J. J., and Scott, W. J. Merle, "The Measurement of Sympathetic Vasoconstrictor Activity in the Lower Extremities," *J. Clin. Invest.*, in press.

normal vasodilatation level. Having established the response to known regional sympathetic paralysis, we now report the effect of certain general anesthetics upon the vasoconstrictor gradient in order to simplify if possible the methods for gaining this information.

We have found that nitrous oxide-oxygen, ether, and tribromethyl alcohol individually in anesthetic doses will completely obliterate the vasoconstrictor gradient. Thus Chart 1 records the surface temperatures in the toes and soles of a patient, otherwise normal, undergoing an operation for herniotomy under nitrous oxide-oxygen anesthesia. The toes show a rise of 9°C., and the soles of 7°C., both reaching the same level, 34°C. In Chart 2, the temperatures of the left great toe, left sole and left hand are recorded before and after nitrous oxide-oxygen anesthèsia in a boy with right dactylitis. The initial temperature of the toe was 22°C., that of the sole, 25.5°C., and that of the hand 30.5°C. Ten minutes after beginning nitrous oxide-oxygen anesthesia these surface temperatures, originally showing various degrees of vasoconstriction, had begun to increase and 20 minutes after the beginning of the anesthetic they had reached the normal vasodilatation level and were all within 1°C. of each other. Similar results were obtained with ether and tribromethyl alcohol.

If the anesthesia does not reach the proper depth, which we

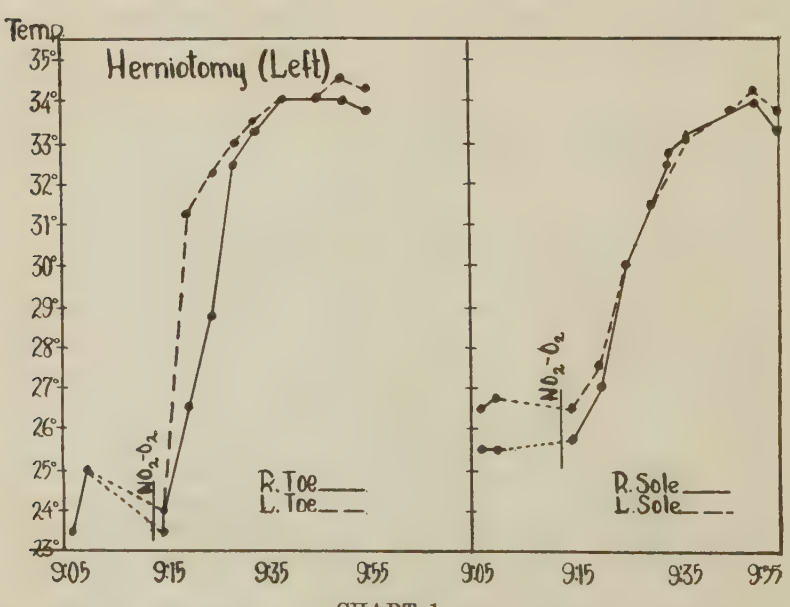

CHART 1.

Normal blood vessels. Vasoconstriction overcome by nitrous oxide-oxygen anesthesia.

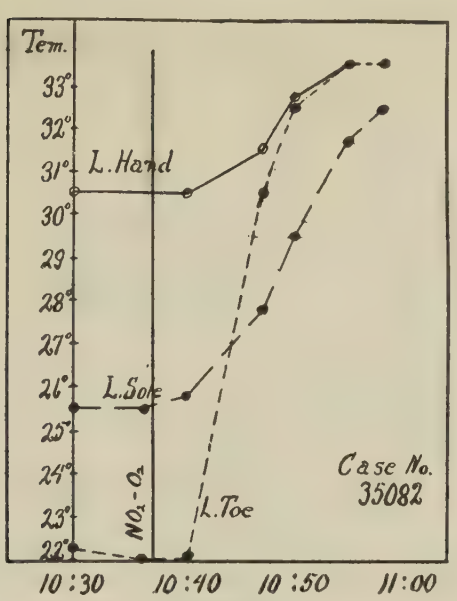

CHART 2.

Normal blood vessels. Obliteration of vasoconstrictor gradient in extremities by nitrous oxide-oxygen anesthesia.

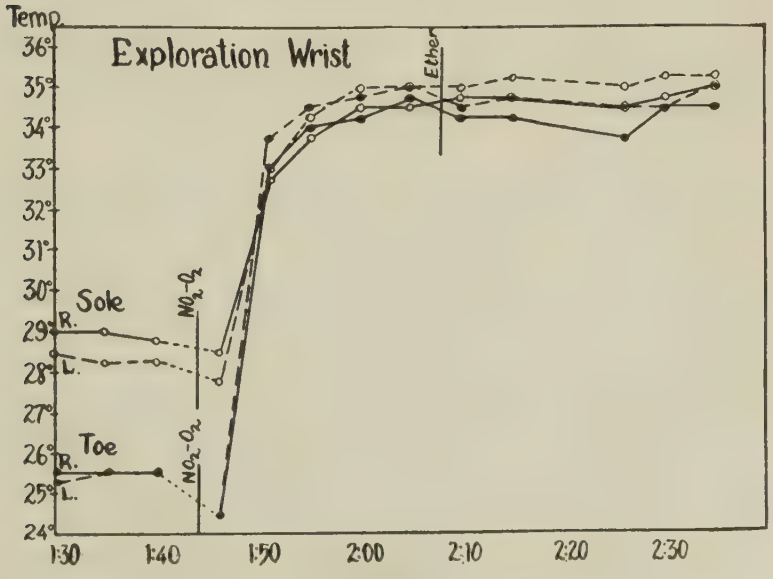

CHART 3.

Normal blood vessels. Normal vasodilatation level produced by nitrous oxide-oxygen anesthesia. Addition of another general anesthetic agent causes no further change in level.

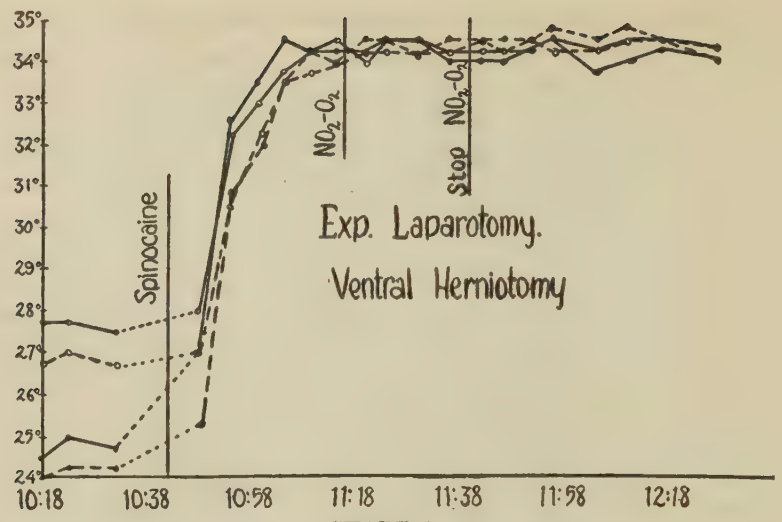

CHART 4.

Normal blood vessels. Normal vasodilatation level produced by spinal anesthesia. Addition of a general anesthetic causes no further change in level.

have associated with loss of consciousness and moderate muscular relaxation, complete vasodilatation does not occur. In fact, with struggling a temporary increase in vasoconstriction may be observed. Usually within 20 minutes of the induction of a satisfactory anesthetic, the gradient is completely abolished. Nitrous oxide-oxygen is satisfactory alone in many cases. In some, it is convenient to add a little ether in order to facilitate complete anesthesia. This in no way vitiates the test.

When the normal vasodilatation level is reached with one of these anesthetic agents, the addition of a second one has no effect on the surface temperature readings. (Chart 3.) Also, after obtaining the maximum effect on the surface temperatures from spinal anesthesia, the administration of an inhalation anesthetic causes no augmentation. (Chart 4.) These phenomena were to be expected on the basis of our hypothesis that the vessels when freed from sympathetic vasoconstriction dilate to their physiological maximum.

An application of these studies to vascular diseases is shown in Charts 5 and 6. The general anesthesia produced by nitrous oxide-oxygen reinforced for relaxation with ether causes fully as complete vasodilatation to the normal level as that which followed spinal anesthesia. At least under certain conditions it is simpler to induce a short inhalation anesthesia than it is to carry out spinal anesthesia

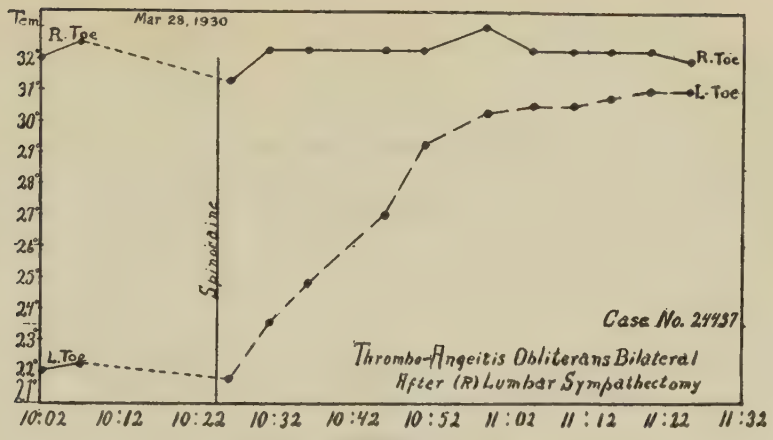

CHART 5.

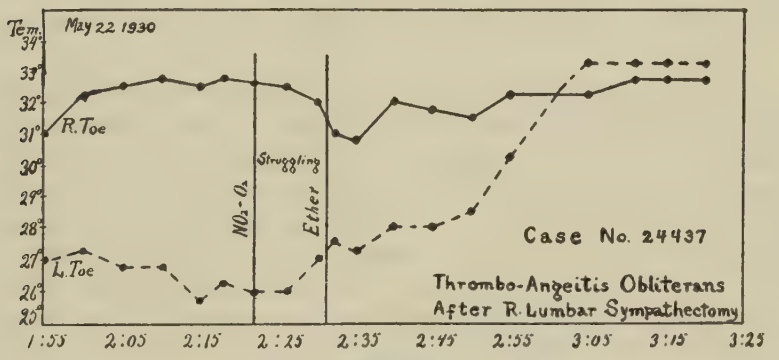

CHART 6.

Thrombo-angiitis obliterans several months after right lumbar sympathectomy. Right extremity at normal vasodilatation level and shows no significant change from either spinal or general anesthesia. Left extremity (non-operated)—

(5) Spinal anesthesia overcomes vasoconstriction.

(6) General anesthesia—Nitrous oxide-oxygen alone; unsatisfactory anesthesia and little effect on vasoconstriction. Nitrous oxide-oxygen reinforced with ether; satisfactory anesthesia and complete obliteration of vasoconstriction.

or paravertebral block.[1, 2, 3] Although the latter are more selective in their action, the estimation of vasospasm in vascular disease seems to be as satisfactory by means of these general anesthetics.

[2] White, James C., *J. Am. Med. Assn.*, 1930, xciv, 1382.

[3] Brill, S., and Lawrence, L. B., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 728.

Minnesota Section.

University of Minnesota, May 28, 1930.

5055

A Parallelism Between Blood Sugar, Blood Calcium and Blood Coagulability in Normal and Jaundiced Dogs.

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(Introduced by Arthur D. Hirschfelder.)

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It has been noted by many early observers, among them Wright,[1] that the coagulation time of blood was decreased after meals. Schreiber[2] and Kehr[3] suggested the preoperative and postoperative use of intravenous glucose in obstructive jaundice, but it was not used extensively until Crile and Walters advocated it 5 years later. Partos and Svec[4] pointed out that a relation existed between the sugar content and coagulability of the blood, namely, that with increasing blood sugar the clotting time is reduced. They demonstrated this effect following the injection of various substances which produce a hyperglycemia, *e. g.*, glucose, epinephrine, theobromine sodium salicylate and morphine. Ravdin[5] presented a series of experiments in which he attempted to justify the use of intravenous glucose in cases having lesions of the biliary apparatus and who were poor operative risks.

In determining coagulation time we used 2 methods simultaneously in all cases, (1) that of Lee and White[6] and (2) a modification of the method of Wright[7] (using pieces of glass tubing 40 mm.

[1] Wright, A. E., *Brit. Med. J.*, 1894, ii, 57.

[2] Schreiber, E., *Centralbl. f. Chir.*, 1913, ii, 1200.

[3] Kehr, H., *Ergebn. d. Chir. u. Orthop.*, 1914, viii, 471.

[4] Partos, A., and Svec, F., *Cerch. f. d. ges. Physiol.*, 1927-8, ccxviii, 209; 1928, ccxix, 481.

[5] Ravdin, S., *J. Am. Med. Assn.*, 1929, xciii, 1193.

[6] Lee, R. I., and White, P. D., *Am. J. Med. Sci.*, 1913, cxlv, 495.

[7] Wright, A. E., *Brit. Med. J.*, 1893, ii, 223.

long and 2 mm. internal diameter). Tests for coagulation were made every half minute. The two methods usually checked within one-half minute; often the results were identical; occasionally a minute's difference was observed, especially in cases where the coagulation time was very long, as in the jaundiced animal. Serum calcium determinations were made by the method of Kramer and Tisdall.[8, 9] Blood sugars were determined, using the Folin-Wu technique.[10] Serum bilirubin was determined by Thannhauser and Anderson's modification[11] of van den Bergh's method. All experiments were done on dogs fasted over night.

Both normal and jaundiced dogs were used. We confirmed the results of previous workers, that an increased blood sugar, produced by the injection of glucose intravenously or by other methods, is accompanied by an increased coagulability. However, the coagulability does not increase simultaneously with the blood sugar, but lags behind for from 15 minutes to one and a half hours; nor does the coagulability decrease with the blood sugar, but in many cases stays up several hours after the sugar content is again normal. It was noted that with increasing time following total obstruction of the common bile duct, it became increasingly more difficult to bring the coagulation time down to normal, although the blood sugar curve became more and more flattened out as jaundice progressed. In a few experiments where the dogs had been without food for longer periods of time than 12 hours, the blood sugar rose only slightly after intravenous injection of glucose, returned to normal or subnormal very quickly, and the effect on the coagulation time was less. In well fed jaundiced dogs, however, an injection of 50-75 cc. of 50% glucose brought the coagulation time down to well within the normal range and maintained it there for several hours.

No rational explanation has been offered for the mechanism by which an increase in blood sugar reduces the coagulation time of the blood. We have found, however, that the intravenous injection of glucose in sufficient amounts and concentration causes a rise in the blood calcium, as well as an increased coagulability. This increased coagulability seems to parallel the blood calcium curve more closely than that of the blood sugar, as the accompanying table will show:

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[8] Kramer, B., and Tisdall, F. F., *J. Biol. Chem.*, 1921, xlvii, 475.

[9] Tisdall, F. F., *J. Biol. Chem.*, 1923, lvi, 439.

[10] Folin, O., and Wu, H., *J. Biol. Chem.*, 1920, xli, 367.

[11] Thannhauser, J. S., and Anderson, E., *Deut. Arch. f. Klin. Med.*, 1921, cxxxvii, 179.

TABLE I.
Normal dog No. 5, wt. 19 kg., fasted over night.

Time	Coagulation Time		Blood Sugar	Serum Calcium
	Lee and White	Wright		
Normal	11 min.	11 min.	76 mgm. %	10.0 mgm. %
Injection of 75 cc. of 50% glucose intravenously in 15 Min.				
5 min.	8 min.	8 min.	230 mgm. %	10.2 mgm. %
15 "	4 "	4 "	168 " "	13.6 " "
30 "	5½ "	5 "	102 " "	14.2 " "
1 hr.	4 "	4 "	72 " "	13.4 " "
1½ "	4 "	4 "	74 " "	12.6 " "
2½ "	3 "	3 "	68 " "	12.4 " "
3½ "	4 "	4 "	68 " "	11.7 " "

The injection of hypertonic sodium chloride (isotonic with 50% glucose) produced a moderate rise in blood sugar, a rise in blood calcium over a period of several hours and a corresponding decrease in the coagulation time of the blood.

We are led to conclude, therefore, that the reduction in the coagulation time of the blood following the intravenous injection of glucose or of substances which produce a hyperglycemia, is accompanied by an increase in the blood calcium which persists after the blood sugar has returned to normal and closely parallels the coagulability of the blood.

5056

Evaluation of X-Ray Evidence as a Criterion of Strangulation Obstruction.

R. O. GOEHL, F. W. LYNCH, C. BORMAN AND O. H. WANGENSTEEN.

From the Department of Surgery, University of Minnesota.

Recently Wangensteen and Lynch[1] indicated that the accumulation of gas in the small intestine as visualized by X-ray examination was an early and reliable criterion of obstruction to the continuity of the bowel. In this study an attempt has been made to evaluate the significance of X-ray evidence in the early recognition of strangulation obstruction.

Strangulation obstruction in dogs was established under aseptic conditions employing local anesthesia (procaine) of the abdominal wall fortified by the preliminary injection of morphine sulphate.

[1] Wangensteen, O. H., and Lynch, F. W., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 674.

In each instance about 24 inches of small intestine together with its mesentery was tied off about 18 inches above the ileocaecal valve. Umbilical cord tape was used for the tie and an attempt was made to establish varying grades of strangulation which might be designated on the basis of I-IV, IV being the maximal grade of strangulation obtained. In the grade IV strangulations, the blood supply to the loop was arrested; in grade III there was immediate congestion and discoloration of the bowel but slight pulsation could still be felt in the mesenteric loop beyond the ligature after placement of the tie. It was found to be extremely difficult to establish strangulation obstructions of grades I and II, and these experiments concern strangulation obstructions of grades III and IV. In 2 dogs mild (Grade I and II) strangulation obstructions were established; these survived the procedure and subsequent laparotomy showed the continuity of the bowel uninterrupted despite the angulation of the loop. Twelve animals in which grade III and IV strangulations were established serve as the basis for this report. Perforated lead shot were sewed to the mesenteric border of the strangulated loop in an effort to determine whether the bowel proximal to the obstruction or the strangulated loop distended most readily.

In summarizing briefly the results of this study it was found that gaseous shadows were observed in the small intestine fairly early during the course of the obstruction. Employing the same criteria in a previous study of simple obstruction it was found that gaseous shadows were observed proximal to the obstruction in 4 to 5 hours after the establishment of the obstruction. In this series, gas was first observed in the small intestine 3 hours after the establishment of strangulation obstruction and the longest interval was 8 hours, the average interval being 6 hours.

It is an interesting fact that the strangulated loop frequently did not exhibit gaseous distension in excess of that exhibited by the bowel proximal to the obstruction. During the latter course of the obstruction, the distension of the strangulated segment could usually be traced out with ease on the X-ray film. In several of the animals, gaseous distension of the intestine distal to the obstructed loop was also observed, an occurrence not noted in the study of simple obstruction. This finding is probably significant of the concomitant occurrence of inhibition ileus as seen in the paralytic ileus of peritonitis in which the entire intestinal canal participates in the distension.

A number of years ago Wahl[2] described "Darmsteifung" of the

[2] Wahl, E., *C. f. Chir.*, 1889, xvi, 153.

strangulated loop and it has generally been accepted that early distension and stiffening of this coil was the usual occurrence. Rabwin[3] has recently referred to 2 patients with strangulation obstruction in which gaseous shadows failed of demonstration on the X-ray film.

The results of this study indicate that the X-ray is not of the same value in detecting the presence of strangulation as it is in the early recognition of simple obstruction of the intestine.

5057

Observations on the Transfusion of Portal Blood From Dogs With Intestinal Obstruction to Normal Recipients.

H. A. CARLSON, F. W. LYNCH AND O. H. WANGENSTEEN.

From the Department of Surgery, University of Minnesota.

In recent years evidence has accumulated that seriously questions the almost universally accepted belief that the absorption of a toxin from the intestinal canal is responsible for the death of the animal with simple obstruction of the bowel. There exist, however, a few bits of evidence which would substantiate such a belief. Sugito[1] found that blood serum obtained from obstructed dogs when injected into the peritoneal cavity of rats provoked toxic symptoms. Scholefield[2] has obtained results of a similar nature, but it is a noteworthy fact that he found no evidence of a toxic substance in the portal blood until the dogs were in a moribund condition.

In this study, 6 dogs were obstructed by severing and inverting the ends of the bowel in the lower ileum under aseptic conditions. When it was apparent that the obstructed animal was rapidly failing, the abdomen was opened under ether anesthesia and a large mesenteric vein was divided and the portal blood collected into a 3% solution of sodium citrate, (10 cc. per 100 cc. of blood). The blood thus obtained was injected into the external jugular vein of a normal anesthetized dog under aseptic conditions and the blood pressure of the recipient was registered by means of a cannula introduced into the carotid artery. Two other experiments were employed as controls. In one of these, 220 cc. of blood was obtained from the

[3] Rabwin, M. H., *Am. J. Surg.*, 1929, vii, 656.

[1] Sugito, S., *Mitt. a. d. Med. Fak. d. k. Univ. Kyushu. u. Fukuoka.*, 1924, ix, 229.

[2] Scholefield, B. G., *Guy's Hospital Reports*, 1927, lxxvii, 160.

carotid artery of a normal dog and collected in the citrate solution. To this citrated blood 10 mg. of histamine dichloride was added. In the other control experiment, 100 cc. of 1% sodium citrate solution was administered intravenously without the addition of the blood.

The transfusion of the blood from the normal donor to which histamine was added resulted in an immediate and protracted drop of blood pressure to about one-half of the original reading despite the increase in blood volume; 17 minutes later the blood pressure almost regained the initial normal level. The injection of the citrate solution without the addition of the blood resulted in a slight elevation of the blood pressure of the recipient.

In all of the 6 animals transfused with the portal blood of 6 other dogs dying of simple obstruction of the intestine a definite increase of blood pressure was obtained soon after the transfusion was begun. This elevation in blood pressure was sustained for several minutes and then there followed a gradual decline to the normal level. In no instance was there a decrease in pressure following the transfusion of blood. The rise in blood pressure obtained in these experiments is undoubtedly a plethora effect.

Upon completion of the tracings the dogs were returned to their cages and observed for evidence of ill effects. Five of them displayed no unusual symptoms. The last dog in the series, however, died 40 hours after the transfusion. Post mortem examination showed some congestion of the lungs, liver, and spleen and a purulent infection of the wound in the neck, which finding would be adequate cause for the lethal outcome.

TABLE I.

Experiment	Infusion	Amount transferred (cc.)	Days duration of obstruction in donor	Effect on blood pressure in recipient	Result
1	Citrated portal blood from obstructed dog	75	—	Slight elevation	Recovery
2	Same	100	—	" "	"
3	"	200	7	Moderate elevation	"
4	"	200	5	" "	"
5	"	250	3	" "	"
6	"	250	10	" "	Died 40 hrs. later of infection
7	Citrated normal blood plus 10 mg. histamine dichloride	220	No obstruction	Marked depression	Recovery
8	1% sodium citrate solution	100	"	Slight elevation	"

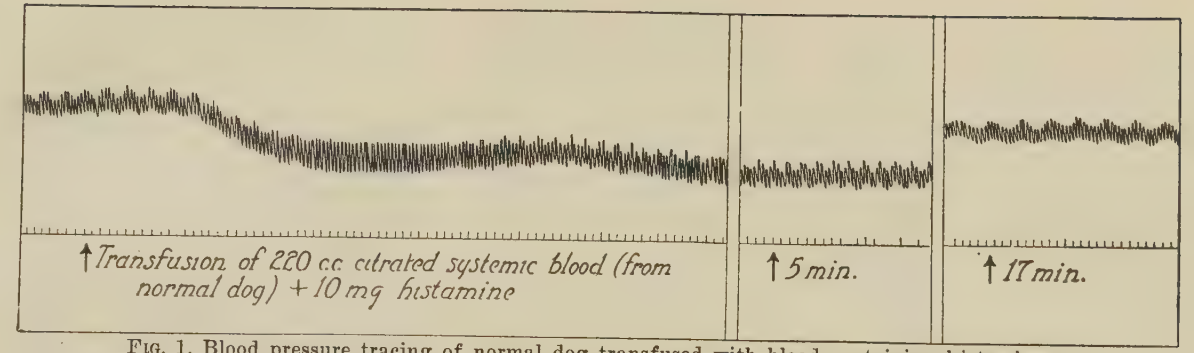

FIG. 1. Blood pressure tracing of normal dog transfused with blood containing histamine.

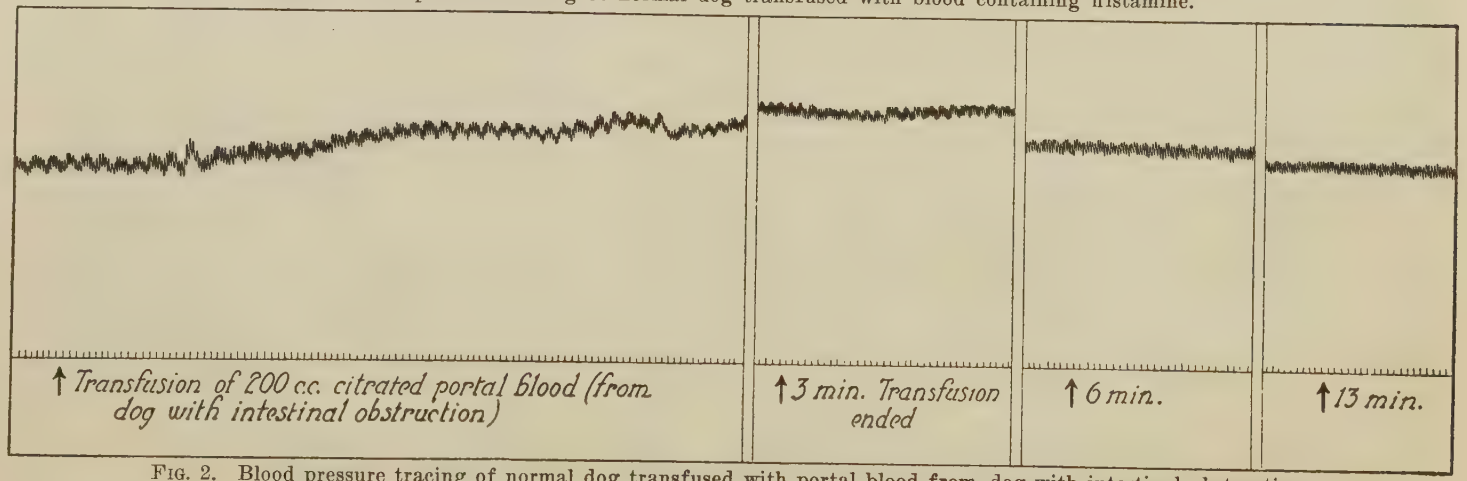

FIG. 2. Blood pressure tracing of normal dog transfused with portal blood from dog with intestinal obstruction.

McLean and Andries[3] and Werelius[4] have previously failed to elicit toxic effects in transfusing normal dogs with systemic blood from dogs with intestinal obstruction. Wangensteen and Loucks[5] were unable to detect evidence of histamine absorption when histamine dichloride was placed in the obstructed bowel. It is a significant fact that in the experiments reported here that portal blood of dogs dying of intestinal obstruction failed to exhibit the physiologic test for histamine; the addition of 10 mg. of histamine dichloride, however, produced immediate and protracted depression of blood pressure.

5058

Observations on Intestinal Obstruction Following the Intravenous Injection of Particulate Material.

OWEN H. WANGENSTEEN AND H. HAMILTON COOKE.

From the Department of Surgery, University of Minnesota.

The cells which are grouped together under the name of the reticulo-endothelial system phagocytose particulate material and destroy or neutralize the action of many soluble toxins present in the circulating blood. The present study was made to observe if a partial blockage of the phagocytic ability of the reticulo-endothelial system had any effect on the length of time an animal would survive following the production of a complete obstruction in the distal portion of the ileum.

Rabbits were selected as most suitable for these experiments. All surgical work was performed under ether anesthesia and with sterile technic. Routine necropsy examinations were made on each animal. To determine the length of time a normal rabbit would live following the production of a complete obstruction in the distal portion of the ileum the following preliminary experiment was performed: In a series of normal rabbits which weighed between 1200 and 3100 gm. a complete obstruction was made by placing a tie of tape 10 cm. proximal to the ileo-cecal valve. Three rabbits lived from 40 to 50 hours; 3 lived from 60 to 70 hours, and 4 lived from 70 to 89 hours after the operative procedure. Forty-four hours

[3] McLean, A., and Andries, R. C., *J. Am. Med. Assn.*, 1912, lix, 1614.

[4] Werelius, A., *J. Am. Med. Assn.*, 1922, lxxix, 535.

[5] Wangensteen, O. H., and Loucks, M., *Arch. Surg.*, 1928, xvi, 1089.

was the shortest period while 89 hours was the longest period any animal survived the operation. The average length of life was 65.8 hours. A second series of 11 normal animals which weighed between 1150 and 3000 gm. were selected. A colloidal preparation of graphite was made and injected intravenously at daily intervals. To avoid the formation of emboli the first injection consisted of as small quantities as 0.5 cc. of the graphite preparation. This amount was gradually increased until 10 cc. was injected at one time. The total quantity injected into the animals varied from a minimum of 32 cc. to a maximum of 105 cc. After certain amounts of graphite had been injected the animals were not disturbed for 2 days. A complete obstruction was then produced in the distal ileum 10 cm. proximal to the ileo-cecal valve.

TABLE I.
Length of Life Following the Production of Intestinal Obstruction in Rabbits with Partial Blockage of the Reticulo-endothelial System.

Number	Sex	Weight in gm.	Total quantity of graphite injected intravenously	Quantity of graphite injected per kilo body wgt.	Length of life in hours after the production of intestinal obstruction
1	F	3000	32	9	26
2	M	1700	35	20	21
3	F	1650	35	21	29
4	F	1300	35	26	34
5	M	1500	50	33	12
6	M	1350	55	34	20
7	M	1550	55	35	21
8	M	1900	105	52	7
9	F	1400	105	69	6
10	F	1300	105	75	6
11	F	1150	105	90	5

The length of survival following the production of complete obstruction 10 cm. from the ileo-cecal valve was definitely shorter among 11 rabbits with a partial blockage of the reticulo-endothelial system than among normal rabbits. The shortest time a rabbit with partial blockage of the reticulo-endothelial system lived after the production of complete obstruction in the distal portion of the ileum was 5 hours and the longest period 26 hours. The average duration of life in this group was considerably less than among the normal animals. There appeared to be a relation between the quantity of particulate material ingested by the cells of the reticulo-endothelial system and the length of survival following the production of intestinal obstruction.

5059

Have the Adrenal Glands a Specific Detoxifying Function in Intestinal Obstruction?

OWEN H. WANGENSTEEN AND H. HAMILTON COOKE.

From the Department of Surgery, University of Minnesota.

The adrenal glands perform a number of functions which appear necessary for normal mammalian life. Some animals, especially rats and rabbits, are, however, able to survive bilateral adrenalectomy for several weeks. Many interesting experiments have been performed to show that the adrenal glands secrete a substance or substances which act as a special defensive agent in the destruction of various types of toxins. The detoxifying substances are believed to be elaborated principally by cells of a reticulo-endothelial nature which are located in the cortex of the gland.

The cause of death in low intestinal obstruction is not well known. Cutting[1] has, in a recent article, presented experimental evidence which seems to show that a material elaborated in the adrenal cortex neutralized or destroyed a toxin or toxins responsible for the effects of intestinal obstruction. The adrenal glands were, therefore, considered to act as a specific mechanism against a toxemia present in all cases of advanced intestinal obstruction. These deductions stimulated us to perform a group of experiments for the observation of the effects produced by the intravenous injection of a toxic material into animals on whom various types of operations had been performed. All surgical procedures were done with sterile technic and ether anesthesia. Bilateral adrenalectomies, unilateral adrenalectomies, cholecystectomies, unilateral nephrectomies, splenectomies and appendectomies were done on a group of 32 rabbits. Ten of the animals survived the operative procedure and appeared well 3 weeks later.

Complete intestinal obstruction was produced in a group of 10 rabbits by placing a double tie of linen tape 10 cm. proximal to the ileo-cecal valve. As soon as an animal died a sterile material was prepared from the fluid proximal to the point of obstruction. The technic for the preparation of this material has been described elsewhere by one of us.[2] The material obtained from each animal was then divided into 2 equal portions. One-half was injected intravenously through the ear vein into an animal on which a surgical proced-

[1] Cutting, R. A., *Arch. Surg.*, 1929, xix, 272.

[2] Wangensteen, O. H., and Chunn, S. S., *Arch. Surg.*, 1928, xvi, 606.

ure had been performed and one-half into a normal healthy animal which served as control. An effort was made to obtain as a control an animal which weighed more than one on which an operation had been performed. The results have been tabulated.

TABLE I.
The Effects Produced by the Intravenous Injection of a Toxin Material on Animals on Which Various Surgical Operations Had Been Performed.

Number	Sex	Weight in gm.	Type of Operation	Quantity of toxin injected intravenously in cc.	Degree of reaction following intravenous injection	Length of life after injection of toxin
Ex. 1	M	1725	Bilateral adrenalectomy	60	Marked	24 hrs.
Con. 1	M	1800	None	60	"	Died 15 min.
Ex. 2	F	1650	Left adrenalectomy	45	"	Well 2 days
Con. 2	F	1680	None	45	Moderate	10 hrs.
Ex. 3	F	2000	Right adrenalectomy	32	Slight	14 hrs.
Con. 3	F	2100	None	32	Marked	18 hrs.
Ex. 4	M	1410	Cholecystectomy	55	Moderate	Well 7 days
Con. 4	M	1470	None	55	"	21 hrs.
Ex. 5	M	1945	Cholecystectomy	40	"	14 hrs.
Con. 5	M	1990	None	40	Marked	2 hrs.
Ex. 6	M	2340	Right nephrectomy	35	"	Well 2 days
Con. 6	M	2425	None	35	Moderate	26 hrs.
Ex. 7	F	1870	Left nephrectomy	47	Marked	4 hrs.
Con. 7	F	1900	None	47	Slight	10 hrs.
Ex. 8	M	2125	Splenectomy	65	Marked	20 hrs.
Con. 8	M	2200	None	65	Moderate	29 hrs.
Ex. 9	F	1360	Splenectomy	54	Marked	22 hrs.
Con. 9	F	1420	None	54	"	46 hrs.
Ex. 10	F	1245	Appendectomy	36	Moderate	Well 8 days
Con. 10	F	1380	None	36	Slight	15 min.

Each pair of animals was ill after the injection of the material. The effects usually consisted of listlessness, weakness, tremors, spastic muscular contractions, rapid respiration, increased pulse rate, dilatation of the pupils and lowering of the blood pressure. A few animals developed a temporary comatose condition and 2 died soon after the injection. The degree of reaction varied considerably both among the animals which had been operated upon and among the controls. It is interesting to note that 7 of 10 animals on which operative procedure had been performed lived longer following the injection of the toxin than the normal animals which served as control. All 3 of the animals on which one or both of the adrenal glands had been removed lived longer than the control animal.

Deductions are difficult to make from this small series but it ap-

pears that following any major operative procedure the entire reticulo-endothelial system is mobilized for the defense of the body against the action of toxic material. The relatively few reticulo-endothelial cells found in the cortex of the adrenal gland do not appear to produce a specific substance for the defense of the body against the toxin produced in intestinal obstruction.

5060

The Effect of Ether and Chloroform on Kidney Function in Dogs With Obstructive Jaundice.

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From the Department of Surgery, University of Minnesota.

Clairmont and Haberer[1] first directed attention to the terminal anuria that occasionally accompanies protracted obstruction of the common bile duct. This happening has frequently been observed since then but the mechanism of its occurrence is not well understood. It has been believed that the administration of ether anesthesia to patients with obstruction of the common bile duct has played a significant rôle in precipitating this event.

In this study we had planned to determine whether any of the commonly employed anesthetics have a deleterious influence on the kidney function in the presence of obstructive jaundice. Not having obtained any manifestations of such injury with chloroform or ether no further observations were made with other anesthetics in common use.

The common bile duct was divided and ligated in 16 normal dogs under aseptic conditions. Following the convalescence of the animal, deep ether anesthesia was administered for an hour at brief intervals (usually a week apart) following which the excretion of phenol-sulphon-phthalein and the blood urea and icterus index were obtained on 2 occasions soon after recovery from the anesthetic and again before its repetition a week later. In a few other dogs similarly obstructed, chloroform was administered in one instance by stomach tube and to 4 others by inhalation.

Blocks of tissue from the kidney and liver were obtained at necropsy from all the dogs that served as subjects of these experi-

[1] Clairmont, P., and Haberer, H., *Mitt. u. d. Grenzgeb. d. Med. u. Chir.*, 1911, xxii, 159.

ments. The kidneys exhibited typical tubular degeneration; in most instances this finding was striking. Fat stains made of the kidney showed a corresponding fatty degeneration of the tubules. Biliary pigment and albumin were usually observed within the lumen of the tubules. Bile pigment was also found deposited within the renal cells. In the liver marked central necrosis of the lobules with fairly normal periportal spaces were seen. Only in one instance was there evidence of a proliferating process suggestive of cirrhosis in the periportal spaces. In this instance the central necrosis was minimal. Fat stains demonstrated marked fatty degeneration of the liver in all specimens.

TABLE I.
Dogs With Common Bile Duct Obstruction by Ligation and Division.

Dog number	Survival in days	$CHCl_3$ Administration			P. S. P. Output		Blood urea nitrogen		Icterus index. Highest value reached
		By stomach tube. No. times given. Each time 10 cc.	By inhalation. No. times given 10 cc.	Ether by inhalation 1 hr. No. times given	Preoperative %	Postoperative %	Preoperative. Mg. per 100 cc. blood	Postoperative. Mg. per 100 cc. blood	
1	54	9		4	80	80-100	10.26	8.0 -10.26	72
2	5			1	90		15.86	24.64*	
3	84			3	50	40-50	16.8	8.4 -18.9	18
4	83			3	35	20-90	12.13	13.06-17.02	24
5	13			2	60-65	50			
6	27			3	50-60	35-50		71.85*	128
7	9			2	80	45-80		51.26*	128
8	59			7	55-85	45-85	16.8 -21.1	14.3 -19.01	112
9	87			7	50-60	55-70	19.6	13.2 -19.1	80
10	51			6	45-60	50-80	14.1	18.13	48
11	14			1					
12	36		1	1	80	70			
13	3			1	55				
14	8		1	1	85	85	22.4		
15	31		3	1	70	70-80	24	10.2	112
16	26+		4	1	80	45-60		23.5	

* Terminal.

Briefly it may be stated that no evidence was obtained to indicate that the administration of ether or chloroform impaired the kidney function in an animal with complete obstructive jaundice. The phenol-sulphon-phthalein excretion remained normal up to the end, and in 2 instances only was a terminal increase in the blood urea noted.

5061

The Rôle of the Fatty Acid Compounds of the Phagocytes in Neutralizing Bacterial Toxins.

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From the Department of Bacteriology and Immunology, University of Minnesota.

The rôle of the phagocytes in the mechanism of resistance to infection has been recognized since the early work of Metchnikoff in this field. Little work, however, has been published on the fate of the toxins of the ingested bacteria. The present study was undertaken with the view of determining the rôle of fatty acid compounds of the phagocytes in neutralizing diphtheritic toxin.

Pus from various types of abscesses was studied, particularly with reference to its ability to neutralize diphtheritic toxin. One gram of pus, it was found, would neutralize from one to 4 M.L.D's of toxin, depending upon the nature of the material and the time of contact. The pus was then dessicated and extracted with alcohol and ether in order to remove the fatty acid compounds. The residue, it was found, possessed no neutralizing properties whatever. The extracted fatty acids which were chiefly of the oleic series, when saponified, neutralized diphtheritic toxin in ratios comparable to those published in earlier work from this laboratory.[1]

It is not only the salts of the unsaturated fatty acid series which neutralize toxins. Some of the esters, such as lecithin, cholesterin, and kefalin also possess active detoxifying properties. It seems probable, therefore, that the fatty acid compounds of the body play an important rôle in the mechanism of resistance to bacterial toxins.

[1] Colloid Symposium Monograph, iii, 152.

5062

Experiments Leading to a Possible Basis for Vaccine Therapy in Acute Rheumatic Fever.

B. J. CLAWSON AND G. E. FAHR.

From the Department of Pathology, University of Minnesota, and the Department of Medicine, Minneapolis General Hospital.

The experiments and observations of Swift[1] and Birkhaug[2] showed that there is marked similarity in the hypersensitiveness (allergy) to streptococci in animals made hypersensitive experimentally and in patients having acute rheumatic fever.

With this similarity in mind we have carried on a series of experiments in animals to determine the relation between hypersensitiveness and the concentration of antibodies in the blood.

Rabbits were made hypersensitive to streptococci according to Swift's method by injecting 5 cc. of agar heavily seeded with streptococci into the subcutaneous tissue in one area. In from 12 to 15 days these rabbits were injected subcutaneously with 1/100 of a standard amount of streptococci in each of 10 places on the right side and with 1/1000 of the amount in each of 10 places on the left side. The character, number, and size of these nodules were used as an indicator of the degree of hypersensitiveness of the animals. Some of these hypersensitive animals were injected intravenously with streptococci from 3 to 5 days before the multiple subcutaneous injections were made. The serum of the animals was tested for agglutinins at *the time of the multiple subcutaneous injections and* 5 *days later* when the animals were killed and the nodules examined.

The following facts were observed: 1. In the hypersensitive animals gross lesions, often large abscesses, were frequently seen. 2. The humoral immunity as indicated by agglutinins was relatively low. 3. Giving an intravenous injection of streptococci to these hypersensitive animals, in from 3 to 5 days before the multiple subcutaneous injections in the back, prevented the development of subcutaneous nodules. 4. These desensitized animals showed a high humoral immunity as indicated by a high agglutinating titer. 5. The high humoral immunity appeared to be the factor which prevented the development of subcutaneous nodules.

The fact that hypersensitiveness in rabbits injected with strepto-

[1] Derick, C. L., and Swift, H. F., *J. Exp. Med.*, 1929, xlix, 615.

[2] Birkhaug, K. E., *J. Infect. Dis.*, 1929, xliv, 363.

cocci and agar is similar to the hypersensitiveness in patients with acute rheumatic fever, and the fact that hypersensitive animals may be made nonsensitive and the further progress of the lesions retarded by giving intravenous injections of streptococci led to the inquiry whether patients with acute rheumatic fever, who are known to be hypersensitive to streptococci, might be made nonsensitive, the further progress of the lesions retarded, and the development of new lesions prevented by giving intravenous injections of streptococci.

Eight patients having acute rheumatic fever were injected intravenously from 4 to 9 times with killed streptococci. The initial doses contained from 25 to 100 million organisms and the final doses one billion each. The agglutinating titers of the sera before any injections were made were from 1:0 to 1:400. The final titers following the injections ranged from 1:6400 to 1:100,000.

5063

Effect of Gastrostomy Feedings on Occurrence of Experimental Acute Pancreatic Necrosis After Ampullary Obstruction.

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From the Department of Surgery, University of Minnesota.

Gall bladder disease is a factor common to the majority of cases of acute pancreatic necrosis. The mechanism by which gall bladder disease predisposes to pancreatic necrosis has not been established. In this study we have tried to evaluate the factor of reflux through the agency of mechanical obstruction at the ampulla in the cat. The terminal portion of the common bile duct embedded in the wall of the duodenum was exposed and a ligature placed creating a common channel of the bile and pancreatic ducts.

The criterion of pancreatic necrosis used in this work was the actual histological demonstration of necrosis in the pancreas. Postmortem autolysis and fat necrosis were differentiated from true pancreatic necrosis by the absence of microscopic cellular reaction in these areas.

In a group of 7 cats, 1 developed pancreatic necrosis following simple establishment of ampullary obstruction. In another group air was injected into the gall bladder at the time the obstruction was established until the gall bladder became so distended that its contents were spontaneously evacuated. Through the agency of the

block at the ampulla regurgitation occurred into the pancreatic duct. In a group of 13 cats, 8 developed pancreatic necrosis. The pressure in the common bile duct occasioned by the emptying of the gall bladder was measured in 5 cats and was found to vary between 190 mm. and 240 mm. water.

In another group ampullary obstruction was established and gastrostomy was also done, after which the cats were given frequent feedings of olive oil, cream, and bile salts through the gastrostomy tube. Of 31 cats pancreatic necrosis was observed in 15.

To another group alcohol was fed through the gastrostomy tube. Of 9 cats, 2 developed pancreatic necrosis. In another group frequent feedings of glucose were similarly given. Pancreatic necrosis was observed 2 times in 9 cats. The incidence of pancreatic necrosis in the latter 2 groups is definitely less than in the group given a fat diet.

Of 6 cats treated by gastrostomy feedings of cream, olive oil, and bile salts, but given subcutaneous injections of pilocarpine hydrochloride, 3 developed pancreatic necrosis.

In another group active pancreatic extract was injected into the gall bladder at operation. Pancreatic necrosis was observed once in 3 cats.

In 19 cats in which infections had been established in the gall bladder, pancreatic necrosis occurred in 5. It is to be noted that the incidence of pancreatic is but little greater in this group than in simple ampullary ligation.

In an effort to demonstrate reflux in the pancreatic duct by x-ray, lipoidol was injected into the gall bladder in 6 cats, 1 of these developed pancreatic necrosis. In none of these was x-ray evidence of reflux into the pancreatic duct present.

The more frequent occurrence of pancreatic necrosis in the group in which fatty meals were administered by which emptying of the gall bladder was stimulated, indicates the significance of the emptying of the gall bladder for the development of pancreatic necrosis through the reflux mechanism. It is well known that pancreatic necrosis not infrequently follows large meals in the human.

Mann and Giordano[1] have shown that emptying of the gall bladder causes but little rise in intraductal pressure. However, in the presence of mechanical or functional ampullary obstruction this seems to be a significant factor.

[1] Mann, F. C., and Giordano, A. S., *Arch. Surg.*, 1923, vi, 1.

5064

Effect of Addition of Macerated Tissues on Healing of Granulating Wounds.

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(Introduced by O. H. Wangensteen.)
From the Department of Surgery, University of Minnesota.

Alexis Carrel[1] showed that extracts of several days' old chicken embryos had the remarkable property of accelerating and maintaining indefinitely the *in vitro* growth of fibroblasts. Carrel and Ebeling[2] showed that homologous and heterologous adult organ tissues (muscle, ovary, etc.) also, accelerated the *in vitro* growth of fibroblasts, but did not maintain growth indefinitely. Recent work purporting to show the accelerating effect of chick embryo extracts on wound healing in animals and man has been done by French and German workers.

The purpose of the present work is to investigate the effect on the healing rate of granulating wounds by applying various organ tissues to their surfaces.

Bilateral circular skin wounds extending down to the superficial fascia and varying in size from about 16 to 70 sq. cm. were made on the backs of 14 dogs. The wounds on the right side were used for experimental purposes and to their surfaces the various tissues under investigation were applied every second to fourth day. The wounds on the left side were used for control purposes. These were always dressed with neutral sodium stearate in which was incorporated Chloramine *T* (Carrel and Hartman)[3] for purposes of maintaining sterility. The size of the wounds on both sides was read every second to fourth day. This was done by tracing the edges of the wounds on cleansed, sterile, old, x-ray film. The wounds healed in from about 35 to 72 days. They were arbitrarily considered healed when their area reached the neighborhood of 0.4 sq. cm. The following is a summary of the data observed:

The experimental wounds in dogs 4, 9, 11, and 14 showed a tendency to slightly earlier healing than the control wounds. All other experimental wounds showed retarded healing compared to the control or a healing time equal to it.

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1 Carrel, A., *J. Exp. Med.*, 1913, xvii, 14.

2 Carrel, A., and Ebeling, H., *J. Exp. Med.*, 1923, xxxviii, 499.

3 Carrel and Hartman, *J. Exp. Med.*, 1917, xxvi, 95.

TABLE I.

Dog	Experimental tissue used	*Area at time zero		Area when considered healed		Constants used in formula for prediction		Actual No. days control required to heal	Predicted No. days control required to heal	Difference between actual and predicted No. of days	% error in predicted No. days
		Control	Experimental	Control	Experimental	K	2p				
1	2	3	4	5	6	7	8	9	10	11	12
1	Implantation skin grafts—autoplastic	44.2	42.8	0.3	0.3	.06	200.6	37.0	39.0	2.0	5.4
2	Implantat'n muscle grafts—autoplastic	15.9	16.4	0.4	0.7	.04	89.0	22.0	28.0	6.0	27.0
3	Implantation skin grafts—autoplastic	32.2	33.5	0.4	1.3	.03	159.0	48.0	56.0	8.0	16.6
4	Wolfe-Krause skin grafts—autoplastic	29.7	32.3	0.5	H.	.02	25.7	35.0	38.0	3.0	8.6
5	Spleen pulp—10 day infant	63.4	70.8	0.3	7.4	.03	37.8	49.0	43.0	6.0	12.2
6	Wolfe-Krause skin graft—guinea pig	81.4	84.8	0.4	0.5	.06	153.0	52.0	38.0	14.0	27.0
7	Spleen pulp—calf fetuses, 6-7 months	64.6	64.6	0.5	0.8	.02	8.4	48.5	35.0	13.5	27.8
8	Same as 7	41.6	37.3	0.2	1.8	.02	11.6	45.5	40.5	5.0	11.0
9	Fetal dog liver	65.6	65.0	0.3	0.1	.03	25.4	27.0	41.9	5.1	11.0
10	Same as 11	75.4	75.2	0.6	1.3	.02	18.7	46.0	44.0	2.0	4.3
11	Fetal dog kidney	64.5	66.5	0.6	H.	.04	50.0	47.5	35.0	12.5	26.3
12	Implantation fetal dog skin graft	84.3	93.5	2.0	3.0	.04	62.1	40.0	30.8	9.3	23.1
13	Liver extract—Stearn's	50.3	55.5	0.2	0.3	.05	24.7	45.0	36.0	9.0	20.0
14	Fetal calf liver—powdered	81.7	98.2	1.6	1.4	.03	39.0	48.5	33.5	15.0	31.0
										Average error in prediction for series	18.0

*Area at time zero = starting point for the calculated predictions and not necessarily the original size of the wound, which may have been slightly larger or smaller. H = healed.

In 1919, Lecomte P. duNoüy[4] formulated a general equation for the law of cicatrization of surface wounds:

$$\text{Log } S_t = \text{Log } S_o - K\left(T - \frac{T^2}{2p}\right)$$

$$K = \frac{\text{Log } S_o - \text{Log } S_t}{T \text{ (4th day)}}$$

$$A = \frac{\text{Log } S_o - \text{Log } S_t \text{ (8th day)}}{K} - T \text{ (8th day)}$$

$$2p = \frac{T^2}{A}$$

Using the above formula the time of healing of each control wound was predicted by calculation.

Column 12 of the table (% of error in predicted days) shows that the predictions were correct with errors varying from 4.34% in dog 10 to 31.0% in dog 14. The average error in prediction for the whole series of 14 dogs was 18%.

The causes of error in prediction were chiefly infection of the wound, due to dislodgement of the wound dressing by the dog.

Conclusions. 1. No tissue investigated caused any striking accelerating of healing. 2. The healing time of the control wounds was predicted by calculation with an average error of 18% for the whole series.

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[4] du Noüy, Lecompte P., *J. Exp. Med.*, 1919, xxix, 329.

Illinois Section.

Billings Hospital, May 27, 1930.

5065

Calcium Partition in Blood Serum in Vascular Hypertension and in Experimental Hypervitaminosis D.

SMITH FREEMAN AND CHESTER J. FARMER.

From the Department of Chemistry, Northwestern University Medical School, Chicago, Illinois.

Partition of serum calcium in 16 cases of vascular hypertension without treatment showed the average value for diffusible calcium to be 57% of the total serum calcium. Determinations carried out on 5 apparently normal individuals of similar ages gave an average value of 65% for diffusible calcium. The average total serum calcium for both groups was almost identical, being 10.3 mg./100 cc. for normals, and 10.5 mg./100 cc. for hypertension cases.

In hypervitaminosis D, produced in adult rabbits by feeding large doses (26,000 to 61,000 rat units per day) of irradiated ergosterol dissolved in sesame oil,[1] the percentage of diffusible calcium decreased from an average normal value of 58% to 49% of the total serum calcium. The average total serum calcium in these animals increased from 14 mg. to 17 mg. per 100 cc. of serum. Inorganic phosphorus values in this condition increase to approximately twice the normal value. Serum proteins remained unchanged in hypervitaminosis D, the average normal value being 6%.

Daily doses equal to or greater than 26,000 rat units of irradiated ergosterol were found to be definitely toxic for adult rabbits weighing from 2 to 3 kg., if administered for 15 days or longer.

[1] Supplied through the courtesy of the Abbott Laboratories.

5066

Heart Rhythms in Frog and Turtle as Affected by Ultraviolet Point Radiation.*

MARIE A. HINRICHS AND PHILIP O. C. JOHNSON.

From the Department of Physiology, University of Chicago.

The apparatus used in these experiments has been previously described in a paper published in this journal,[1] and consists of a Cooper-Hewitt quartz mercury-vapor arc as the source of radiation, running at 110 volts D. C. A quartz rod placed at right angles to the axis of the arc, and pointed and bent at a slight angle served to conduct the radiation from the arc to the desired point on the heart's surface. As previously reported for Fundulus hearts, it was possible to obtain an increase in rate of heart beat, or a decrease in rate depending on the length of the period of exposure.

Short exposures in the region of the sino-auricular node of both frog and turtle produced a noticeable increase in rate. When the exposure is too long continued, a decrease in rate follows, and the normal sequence of auricle and ventricle beats may be interfered with. In this connection, further experiments are being done and will be reported in a later paper. Experiments with ultraviolet radiation and heart-block are also under way.

It has been possible to stimulate the heart of a frog to regular beat, normal in sequence and in amplitude, by radiation at the sino-auricular node after the heart has been quiescent for an hour or more. Whether this be a question of stimulation of the pace-making region to greater activity so that the magnitude of the impulse is great enough for conduction through the heart tissue, or whether the pacemaking region is entirely quiescent and is at once stimulated to activity by the exposure, remains to be determined.

* This investigation was supported in part by a grant from the Radiation Fund of the National Research Council, and in part by a grant from the Rockefeller Foundation to the University of Chicago.

[1] Hinrichs, PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 354.

5067

The Determination of the pH of Blood Serum with the Quinhydrone Electrode.

MARTIN E. HANKE.

From the Department of Physiological Chemistry, University of Chicago.

Since the work of Cullen[1] and others on the determination of the pH of serum with quinhydrone, some investigators have had difficulty with this method, because of the rapid drifts in potentials. Cullen, Wilson[2] and others have reported that satisfactory quinhydrone serum pH determinations can be made by reading potentials rapidly at noted times and extrapolating in order to obtain the potential at zero time.

In a recent study at this laboratory it was necessary to perform a large number of dog serum pH determinations, and it seemed desirable to check the colorimetric method by an electrometric method. When parallel pH determinations on dog serum were made with the colorimetric method, using the Hastings bicolorimeter[3] and with quinhydrone at room temperature (24° to 26°) it was found that the 2 methods agree to within 0.02 pH. The quinhydrone method is very rapid and convenient, and since it is free from the personal factor of matching colors, it has, in our hands, been more reliable than the colorimetric method. The procedure is briefly as follows:

The electrode vessel consists of 2 ordinary glass tubes which fit snugly one inside the other, a rubber connection which holds the tubes in any desired relative position, and a platinum wire sealed into the inner tube. The inner tube contains mercury through which metallic contact to the platinum wire can readily be made. The outside tube is 8 cm. in length, and 4 mm. in inside diameter, so that its total capacity is about 1 cc., although these dimensions can be varied at will. By moving the inner tube up or down through the outer tube, liquid can be conveniently drawn up or expelled.

For the determination the vessel is filled with a clear saturated solution of quinhydrone in boiled neutral distilled water to the 0.2 cc. mark. Then, at a time noted with a stopwatch, serum is

[1] Cullen and Biilman, *J. Biol. Chem.*, 1925, lxiv, 727; Cullen and Earle, *J. Biol. Chem.*, 1928, lxxvi, 565.

[2] Wilson, D. W., Paper presented before the American Society of Biological Chemists, 1930 meeting, Chicago.

[3] See Clark, "Determination of Hydrogen Ions," 3rd edition, 169-170.

drawn up to the 0.4 cc. mark. A small air bubble, about .01 cc. or less, is also drawn in, which is important for stirring and mixing the 2 solutions. This is accomplished by inverting the tube 6 or 8 times. Liquid junction is made to a saturated calomel cell by clamping the tube upright in a beaker containing saturated KCl. The potential is measured at 30 seconds, and again at 40, 50, and 60 seconds. The drift in potential between 30 and 60 seconds is usually 2 millivolts, and it is assumed that the drift between zero time and 30 seconds would have been one millivolt greater, or 3 millivolts. The calculation is best illustrated by a particular case. If the observed potentials at 30 and 60 seconds are +9 and +11 millivolts, respectively, then it is assumed that at zero time the potential is +9—3 or +6 millivolts. From the conventional constants for quinhydrone at 25°, (saturated calomel), $pH = \frac{.453 - E \text{ observed}}{.059}$. This gives a $pH = 7.58$ at 25°. Assuming a change in pH with change in temperature of —.012 pH per degree, this would become $7.58 - (13.0 \times 0.012 = 7.58 - 0.16 = 7.42$ at 38°.

By using equal volumes of serum and saturated quinhydrone solutions, uniformity in the speed of formation and in the concentration of the quinhydrone solution are insured, and thus a uniform drift in potential is obtained. The drift is less rapid than if serum is saturated with quinhydrone, as Wilson[2] has pointed out, while the pH of the serum is not appreciably altered by this dilution.

5068

Production of Experimental Lobar Pneumonia in the Dog.*

EDWARD E. TERRELL AND O. H. ROBERTSON.

From the Department of Medicine, University of Chicago.

Until the work of Cecil and Blake[1] lobar pneumonia had not been produced constantly and successfully in the lower animals. These authors employed the *Macacus syrichtus,* a Philippine monkey which is apparently more susceptible to the pneumococcus than the other varieties. Their method consisted simply of intra-

* This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

1 Blake, F. G., and Cecil, R. L., *J. Exp. Med.*, 1920, xxxi, 403.

tracheal injections of small quantities of pneumococci. The disease produced and the lesions observed corresponded closely with lobar pneumonia in man. However, the subsequent employment of this method with other species has failed to produce lobar pneumonia apparently because they were more resistant to the pneumococcus than the *Macacus syrichtus* which unfortunately is no longer allowed to be imported into the United States. Others working with the dog have found that in order to produce pulmonary infection with the pneumococcus, not only must the infecting agent be implanted far down in the bronchial tree but the inoculum must be massive. Lamar and Meltzer[2] using this method were successful in producing in some of their animals lobar consolidation and a disease resembling an abortive lobar pneumonia. But the infected animals which lived longer, died within 2 to 4 days with bacteremia and pyemic complications without true consolidation of the lung. Many of their dogs escaped infection. More recently Coryllos and Birnbaum[3] have employed a modification of this method, spraying large quantities of pneumococci culture into the lung through a bronchoscope either with or without subsequent occlusion of the main bronchus. This resulted either in a transient infection or a widespread pneumonia accompanied by atelectasis with an ensuing generalized infection and fatal termination.

Thus the methods previously used in an attempt to produce lobar pneumonia in dogs, not only have failed to induce the characteristics of the disease as seen in humans, but have employed a dosage which is enormously greater than could occur in the spontaneous inception of the disease in man. The results of previous investigation into the state of the circulating antipneumococcus resistance at the time of the onset of the disease[4] led us to infer that the pneumococcus begins its initial growth in the lungs protected from the pneumococcidal action of the blood which in both dogs and humans is considerable. It seemed probable that if pneumococci were implanted in the lungs in a medium which would allow them to grow protected from the pneumococcus killing properties of the blood, the disease might be initiated with relatively small numbers of organisms. In other words, it might be possible to initiate in a general way conditions giving rise to the spontaneous occurrence of lobar pneumonia in man.

After attempting different means we have adopted a method

[2] Lamar, R. V., and Meltzer, S. J., *J. Exp. Med.*, 1912, xv, 133.

[3] Coryloss, P. N., and Birnbaum, G. L., *Arch. Surg.*, 1929, xviii, 190.

[4] Robertson, O. H., Terrell, E. E., Graeser, J. G., and Cornwell, M. A., in press.

which consists in the intrabronchial injection of 0.05 cc. to 0.5 cc. of an 18 hour culture of Pneumococcus Types I or II, suspended in a viscous starch-broth mixture. For this purpose a radio-opaque catheter is inserted under the fluoroscope and 0.5 cc. to 1 cc. of this pneumococcus starch suspension instilled into a small bronchus as near the periphery of the lung as possible. As a rule, within 24 hours typical lobar consolidation of the injected lobe has occurred as evidenced by x-ray and physical findings. The disease runs a febrile course of 3 to 7 days; the pneumonic lesion either remains localized in one lobe or spreads from lobe to lobe. This experimental disease resembles the natural disease in humans in the manner of the spread of the lesion, the localization of the process (pneumococci do not usually invade the blood stream), the immune response, the abrupt termination of the disease by crisis, lysis, or death and the rapid regression of the process after recovery. Animals were killed at different stages during the disease from one hour after injection of the infecting dose to one or 2 days following recovery. The lesion was found to spread evenly and contiguously thru the lobe, the line of the advancing process usually being sharply demarcated from the normal tissue. With the evolution of the disease the lesion progressed through the different stages observed in the human pneumonic lung from an initial marked congestion to red hepatization and finally to a modified gray hepatization. The microscopic pathology was that of a lobar pneumonia from the beginning and in its general characteristics resembled the picture of the human disease. Certain differences from the human pneumonic lungs were observed. These were the greater degree of blood vessel engorgement seen all thru the disease, the smaller amount of fibrin, and the more rapid decrease in size of the resolving lung.

5069

On the Production of Variant Colonies by Certain of the Intestinal Bacteria.

STEWART A. KOSER.

From the Department of Hygiene and Bacteriology, the University of Chicago.

Much of the literature on microbic dissociation has emphasized the occurrence of rough (R) colonies, in contrast to the smooth (S) form, with, on the whole, relatively little mention of other

colony types. In contrast to this, it has been the writer's experience that frequently many variant colonies of different character are encountered.

The results presented in the accompanying table were obtained in connection with a study of factors which might stimulate the process of dissociation. They are typical of many other similar experiments and illustrate the changes in colony form which were found in cultures of certain of the colon-paratyphoid-dysentery organisms. Smooth forms, previously carried through a series of colony isolations, were used for inoculation of the broth or peptone media. The appearance of variant colonies was followed by streaking agar plates of standard composition at intervals. The changes thus represent those occurring in "ageing" cultures.

The change from S to R appeared to be a gradual one and was not accomplished by distinct or abrupt changes from one colony type to another. All gradations in colony form from the typical white homogeneous colonies with even margins, characteristic of the S form, to the flat, dull, dry-looking, coarsely-granular and irregular colonies of the R form were found. Colonies appearing

TABLE I.
Different Colony Forms Obtained from Ageing Broth Cultures.

Time, Days	*Bact. coli* Broth 37°C.	*Bact. coli* 5% Peptone 37°C.	*Bact. aertrycke* Broth 25°C.	*Bact. aertrycke* Broth 37°C.	*Bact. aertrycke* 10% Peptone 25°C.	*Bact. dysenteriae* Sonne 10% Peptone 37°C.
1	100 S*	100 S	100 S	100 S	100 S	100 S
2			100 S	100 S	100 S	100 S
4	100 S	88 S 10 I 2 R	95 S 5? †	80 S 19? † 1 R	99 S 1 I	90 S 10 I
7	65 S 30 I 5 R	60 S 39 I 1 R	86 S 8? † 6 R	35 S 8 1 22? † 35 R	70 S 30 I	40 S 57 I 3 R
10			96 S 4? †	27 S 68? † 5 R	75 S 25 I	
14	85 S 15 I	70 S 15 I 15 R			70 S 30 1	35 S 62 I 3 R
28	80 S 20 I	95 S 5 I	73 S 27? †		70 S 30 1	10 S 40 I 50 R

* Results are expressed in percentages of the different colony types.
† Small adherent, raised, and slightly convoluted colonies. Impossible to classify as either smooth, intermediate or rough forms.

to fall between these two extremes are referred to as intermediate (I) forms in the table. It will be seen that in ageing cultures such colonies were usually present in large numbers and often far outnumbered the true R forms. At times the change went no farther than the intermediate stage and true rough colonies were never seen, though there was nevertheless a decided departure from the typical smooth form.

It should be pointed out that there may be some doubt as to whether all of the colonies referred to as intermediate really represent transition forms whose destination is the R stage, or whether they may not be simply variants from the S type whose ultimate goal, if any, is not the R form.

At times other variant colonies appeared whose characteristics were so obviously different from those of the S, I or R forms that they could not be classed with them. Thus, from older cultures of *Bact. aertrycke* very small, sticky, raised and slightly convoluted colonies were at times obtained. They are designated in the accompanying table by a question mark. When transferred to agar slants and carried through successive daily transfers on agar at 37° C for periods of 30 to 45 days, they gradually returned for the most part to the S type. Also, when similar well-isolated colonies were allowed to age on agar plates they frequently developed a marginal outcropping of S growth. The individual cells comprising these colonies possessed a capsule-like sheath which surrounded the cell and at times appeared to tie together bunches of cells. Otherwise, there was little difference between them and the cells from smooth colonies. On a few occasions large watery mucoid colonies were found.

In Arkwright's communication,[1] which may be said to be responsible in large measure for the recent renewed interest in the subject of variation, he stated "The variety of colonies met with is so great and the differences between them often so indefinite that attention has purposely been directed only to the more obvious characters . . . .". Some of the more recent workers who have followed the interesting though elusive changes included under the term dissociation, have likewise mentioned the occurrence of intermediate or other colony forms, but the study of these forms has usually been subordinated to that of the more striking R type. The purpose of this report is to call attention especially to the large number of intermediate forms and other variant colonies which may be encountered under certain conditions.

[1] *J. Path. and Bact.*, 1921, xxiv, 36.

5070

Conversion of Rough to Smooth Forms of Certain Dysentery Bacilli by Repeated Transfers in Dextrose Broth.

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From the Department of Hygiene and Bacteriology, University of Chicago.

In our study of the factors influencing change in colony form it was found that repeated transfers in dextrose broth were quite effective in causing reversion of R to S forms of *Bact. dysenteriae,* Sonne. A typical experiment is shown in the accompanying table.

In this and in similar experiments with different R isolations of other strains, irregularity in the rate of reversion to the S form was always seen. In some cases S colonies appeared early in the series of dextrose broth transfers and soon entirely replaced the R forms, while in other instances only a few S forms resulted even after a number of transplants. Also, intermediate and other variant colony forms were more rarely encountered here than in the course of the S to R change.

In a number of instances cultures which had undergone the S to R change in broth or peptone solutions and then the R to S reversion in dextrose broth were subjected to comparison with the original or parent S culture of the same strain. The newly converted S forms produced the same uniform turbidity in nutrient broth and similar fermentation results in dextrose, lactose and sucrose broths, and they were agglutinated by immune S serum apparently in as high dilution as was the parent S culture. In 0.85% salt solution, however, the recently derived S forms showed some

TABLE I.

Daily Transfers 37°C.	3 Separate R Isolations *Bact. dysenteriae,* Sonne. Strain number 268.		
At start	100 R*	100 R	100 R
5th	10 R	100 R	20 R
	90 S		20 I
			60 S
10th	1—R	100 R	28 R
	99+S		72 S
15th	100 S	100 R	5 I
			95 S
20th	100 S	97 I	4 I
		3 S	96 S

* Results are expressed in percentages of the different colony types.
S = Smooth, R = Rough, and I = Intermediate between S and R.
Strain 268 was secured originally from the British Type Collection.

tendency to spontaneous agglutination, while the original S culture showed no such tendency.

5071

The Chloride Content of the Tissues.*

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From the Departments of Physiological Chemistry and of Surgery of the University of Chicago.

In normal animals the chloride content of the blood is maintained at 280-320 mg. percent, even under the conditions of a negative chloride balance. Distilled water, injected intraperitoneally in rabbits rapidly acquires a chloride concentration up to 376 mg. percent and becomes isosmotic.[1] Consequently, by the transperitoneal perfusion of distilled water, it is possible to produce an experimental hypochloremia by dialysis.[2] The blood chloride falls and a large amount of chloride, as well as other electrolytes and organic crystalloids, is found in the dialysate. Rabbits thus treated develop muscular fibrillation, tremors and convulsions and die in from 2 to 5 hours. In a series of 13 rabbits so dialyzed, we found an average of 619 mgs., (from 205 to 896 mgs.), of chloride was removed from the animal as determined by analyses of the perfusate. During the corresponding period of dialysis, the average fall in blood chloride was 79 mgs. percent, (from 300 to 221 mgs.). As the blood volume in the rabbits used was empirically about 200 cc., not more than 175 mgs. of the dialyzed chloride could be accounted for as coming from the blood itself. The source of the other 440 odd mgs. of chloride was evidently other body tissues. As a result our attention has been turned to the chloride content of the tissues.

Two of us (C. B. D. and M. E. H.) have recently determined the chlorides in various tissues from the rabbit. As our primary interest at the time lay in the various lipoidal fractions, these analyses were all made upon dried tissues. The animals were killed by bleeding. The desired tissues were quickly removed, cut to shreds and dried in a current of warm air (40°C) for 10 hours.

* A part of this work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

1 Curtis, G. M., *Biochem. Z.*, 1927, clxxxvi, 95.

2 Curtis, G. M., and Pacheco, G. A., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 874.

They were then ground to pass through an 80 mesh sieve and kept in a desiccator until used. After thorough drying they were weighed into an extraction thimble and extracted with hot ether in a Lansiedl apparatus for 20 hours. The ether was then drawn off and the residue extracted for 30 hours with absolute alcohol. Except for a small amount of chloride remaining in the residue in chloride rich tissues, such as blood, practically all of the chloride was found in the alcohol extract. Duplicate analyses were made on each extract, on the tissue residue and on the original unextracted dried tissue. Two methods of chloride analysis were used. The method of Van Slyke[3] gave excellent results with the extracts. In the case of the whole tissue, however, not all the chloride present was recovered. To overcome this loss the method was modified in that the oxidation was carried out in a sealed Carius bomb.[4] The two methods checked well in the analyses of all the extracts. Whole dried tissue analyses were made by the sealed tube method in a large number of cases. These agreed within 7% with the amounts found in the various extracts as determined by the Van Slyke method.

TABLE I.
Chloride Percentage in Dried Rabbit Tissue.

Tissue	7 normal animals	4 animals, given 35 cc. N/10 HCl intravenously	2 animals, given 50 cc. N/2 NaCl intravenously
Fundus mucosa	0.76	1.72	0.64
Liver	0.24	0.27	0.19
Intestine	0.42	0.54	0.56
Kidney	0.73	0.91	1.03
Vol. muscle	0.29	0.22	—
Sm. muscle	0.38	0.65	—
Lung	0.57	0.94	—
Blood	1.34	1.58	1.53

The results of these analyses are presented in Table I. To determine the redistribution of chloride in the tissues after injection, 4 animals were given 35 cc. of N/10 HCl, containing 125 mgs. of Cl, intravenously. Two animals were given 50 cc. of N/2 NaCl containing 875 mg. of chloride, in the same manner. The injections took from 20 to 30 minutes. The animals were killed 5 minutes after the end of the injection. Changes in the distribution of chloride within the tissues are shown in the table.

No analyses of skin or subcutaneous fatty tissue of the rabbit are included in the table. Analyses made by M. E. Hanke on these

[3] Van Slyke, D. D., *J. Biol. Chem.*, 1923, lvii, 523.

[4] Herman, W. K., personal communication.

tissues in the dog show a chloride percentage of from .10 to .15% for the subcutaneous adipose tissue and of from .80 to .90% for the skin.

While the chloride content of the liver is low, yet the size of the liver (18-30 gm. dry weight) emphasizes its importance as a reservoir for chloride. The chloride content of voluntary muscles is also low as compared to some of the other tissues. Owing to their great bulk, however, they must be regarded as an important chloride reservoir. When chloride is dialyzed away from the blood stream in rabbits, it is restored in part from some body source. The tissues which appear to be the more important as such a source are the skin, voluntary muscles and liver.

5072

Variations in the Blood of Rabbits With Age (Birth to Maturity).*

M. M. KUNDE, M. F. GREEN AND E. CHANGNON.

From the University of Chicago, Chicago, Ill.

The total blood volume, red cell count, volume ratio of corpuscles to plasma, reticulocyte count, hemoglobin and size of the red cells of rabbit from 6 hours after birth to 8 months of age have been studied. At birth the R.B.C. are 4 to 5 million per cu. mm.; the Hb is 13 to 16 gm., the volume ratio of corpuscles to plasma and the total blood volume are higher than at maturity. The size of the R.B.C. is 1/3 to 1/2 times larger than the adult R.B.C. The reticulocytes are 25% to 30% of the R.B.C. at birth and 1% to 3% at maturity

* This work has been aided by a grant (Biological Foundation) from the Rockefeller Foundation.

5073

Response of the Anemia in Experimental Hypothyroidism to Liver Therapy.*

M. M. KUNDE, E. CHANGNON, M. F. GREEN AND G. BURNS.

From the University of Chicago, Chicago, Ill.

Rabbits with thyroids removed approximately 3 weeks after birth develop an anemia which simulates clinical pernicious anemia in that the hemoglobin per cell count is relatively higher than in the normal adult. The R.B.C. are also larger than the normal adult R.B.C. Metamyelocytes and poikilocytoses are present. Feeding liver extract (Lilly) to these rabbits increases the R.B.C. and brings the hemoglobin cell ratio back to normal.

5074

Further Studies in Obstructive Pulmonary Atelectasis.*

W. E. ADAMS. (Introduced by E. Andrews.)

From the Department of Surgery, University of Chicago.

In some recent investigative work,[1] two factors were found essential in the experimental production of massive atelectasis of the lung in dogs, *viz.*, A straining type of respiration, and secondly, some form of obstruction to the bronchus of the lung lobe; either total or valvular. In this work an artificial means of obstruction was used, this obstruction being located in the primary bronchus of the lung lobe.

Either total or valvular obstruction, in the presence of a staining type of respiration, was followed by a massive atelectatic condition of the obstructed lung. If a quiet, normal type of respiration was exhibited, no resultant atelectasis was observed. An example was given of complete obstruction of a secondary bronchus, of 6 weeks' duration, with quiet respiration in which no atelectasis occurred.

* This work has been aided by a grant (Biological Foundation) from the Rockefeller Foundation.

* This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

[1] Adams, W. E., and Van Allen, C. M., *Surg. Gyn. and Obstet.*, 1930, l, 385.

In carrying out some experiments with thermal and silver nitrate cautery,[2,3] it was found that cauterization of a bronchus was followed by complete stenosis in from one to 2 weeks. If this stenosis was located in the primary bronchus of the lung lobe, that lobe was found to be completely atelectatic with the secondary bronchi and bronchioles filled with a mucogelatinous material. However, if the stenosis was located in one of the several divisions of the primary bronchus to the lobe, no resultant atelectasis occurred; only a filling of the secondary and tertiary bronchi and bronchioles distal to the stenosis with the mucogelatinous material.

Group A. 4 dogs. Silver nitrate or thermal cauterization of a secondary bronchus of a lung lobe. Sacrificed at the end of 2 to 6 weeks. Results: Total stenosis of secondary bronchus with no atelectasis distal to the stenosis.

Group B. 7 dogs. Silver nitrate or thermal cauterization of the primary bronchus of a lung lobe. Sacrificed at the end of 2 to 6 weeks. Results: Total stenosis of the primary bronchus with complete atelectasis of the lung lobe.

In view of these experiments it appears very probable there are communications between the smaller air passages, or more likely of the air sacs and cells of the many lobules of a lung lobe. It also appears that obstruction of the primary bronchus or all of its divisions is necessary for the production of massive atelectasis of the lung lobe. Furthermore, although a straining type of respiration appeared essential in the production of this condition within 12 to 24 hours, it does not appear to be essential for its production over a longer period of time.

5075

Histologic Observations on Experimental Chronic Gastric Ulcers in Rabbits.

A. N. FERGUSON. (Introduced by Walter L. Palmer.)

From the Department of Anatomy, Northwestern University Medical School, and the Department of Medicine, University of Chicago.

The experimental chronic gastric ulcers in rabbits, to be described, were obtained by making acute lesions in the stomach, which subse-

[2] Adams, W. E., and Livingstone, H. M., *Ann. Surg.*, 1930, xci, 342.

[3] Adams, W. E., and Livingstone, H. M., "Further Studies in Bronchial Injury and Repair," unpublished work.

quently became chronic. An operative procedure was used which consisted in the excision of a piece of the gastric mucosa by means of a certain technic which has been described.[1] A series of chronic ulcers was collected ranging in age from 3 months to a little over 2 years. Large Flemish Giant rabbits were used. These animals usually live to be about 5 years old and an ulcer of 2 years' duration, therefore, occupies a large part of the life cycle of the rabbit. All animals were kept under the same conditions, and fed identical diets composed of hay, oats, carrots and some lettuce. The ulcers were fixed in formol-Zenker solution, serial sections were made, and stained with ordinary and differential stains.

From a histologic standpoint there are 2 main structures in chronic ulcers to be considered, *i. e.,* the margin and the base. The margin is formed by mucosa which is composed of glands that decrease in length and are separated by relatively more connective tissue as the edge is approached. The last glands tend to overhang the ulcer and fall over into the crater. The cells composing these glands are all of one type, the foveolar cells. These are the cells that line the foveolae of normal gastric glands and have been shown to be the ones responsible for the regeneration of epithelium in acute gastric ulcers. (Ferguson.[1]) In chronic ulcers a few of these cells invariably extend from the last marginal glands outward onto the floor of the ulcer. This indicates that the epithelium at the margin, in even the most chronic ulcers, is continually trying to grow out and cover the defect. In some of the ulcers collected considerable epithelium did extend out onto the floor, almost covering it in a few specimens and indicated that healing had occurred.

The base of these chronic ulcers is composed of 2 main layers covered externally by serosa. The first layer, which forms the floor of the crater, may be called the necrotic layer. It contains a great deal of cell debris and nuclei which stain darkly with hematoxylin. This layer changes rather abruptly into the underlying layer of connective tissue. This connective tissue is composed of young fibroblasts as evidenced by their rounded nuclei and rather large amount of cytoplasm which gives off irregular projections. A great many blood vessels are present and many leucocytes, especially eosinophils and plasma cells, are infiltrated into the tissue. The lower part of this connective tissue layer contains more mature cells and some collagen.

Chronic ulcers which show little or no healing have a rather marked crater and also undercutting into the base at the margins.

[1] Ferguson, A. N., *Am. J. Anat.*, 1928, xlii, 403.

The depth of this crater and the amount of undercutting are one index to the chronicity of an ulcer. These factors, together with the necrotic layer in the base, indicate that destruction is occurring on the floor of the base. The young connective tissue, beneath the necrotic layer indicates that reparative processes are being attempted but that destruction is proceeding at such a rate that the uppermost cells do not have time to reach maturity. If these destructive forces are in excess over the reparative processes in the base, destruction of tissue proceeds until finally there is a perforation.

The observations indicate that the essential factors which prevent healing of chronic ulcers are destructive forces which act on the base. These produce so much destruction and necrosis that the waiting epithelium at the margin is unable to gain a foothold on the base and is, therefore, unable to regenerate and cover it.

5076

Influence of Egg White Upon Gastric Secretion.

J. R. FINDER. (Introduced by Lloyd Arnold.)

From the Department of Bacteriology and Preventive Medicine, University of Illinois College of Medicine, and Research Laboratories of the Illinois State Department of Public Health, Chicago.

The influence of various concentrations of bile upon the gastric secretion in dogs with non-leaking fistulous openings in the stomach has been reported.[1] Some phases of the problem of antigenic absorption through an intact body surface covering layer have been reported,[2] and further work is in progress. This report deals with use of egg white as a vehicle to introduce an antigenic substance into the lumen of the small intestine. Bile has been used by Besredka[3] to sensitize or prepare the intestinal mucosa for antigenic absorption in his oral vaccination against the enteric group of infectious diseases. Arnold[4] offered a different explanation for the action of bile than that suggested by Besredka. Bateman[5] received the literature upon the digestibility and utilization of egg white in dogs and man. Raw egg white leaves the stomach more rapidly than other foods.

1 Arnold, L., and Finder, J. G., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 615.

2 Finder, Lash and Simon, PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 368.

3 Besredka, A., "Local Immunization," Williams & Wilkins, Baltimore, 1927.

4 Arnold, L., *J. Hygiene*, 1929, xxix, 82.

5 Bateman, G. W., *J. Biol. Chem.*, 1916, xxvi, 263.

Bateman showed that diarrhea followed the ingestion of certain amount of raw egg white. Arnold[4] showed that raw egg white injected into the duodenum of dogs caused increased permeability of the wall for bacteria.

Four dogs with non-leaking gastric fistulae were used for these experiments. The material placed into the gastric lumen through the

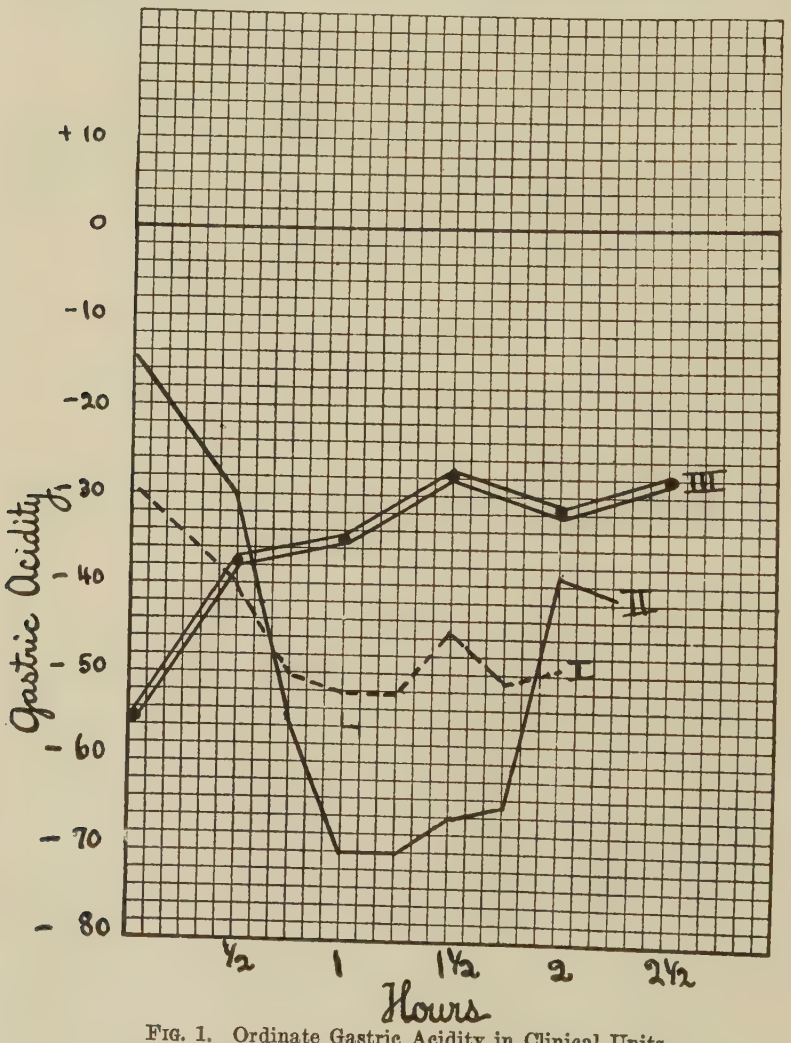

FIG. 1. Ordinate Gastric Acidity in Clinical Units.
Abscissa time in 30 minute periods.

I—Gastric Acidity after raw undiluted egg white.
II—Gastric Acidity after raw diluted egg white.
III—Gastric Acidity after egg white bile mixtures.
Each curve is an average of 15 experiments. (3 animals with 5 experiments on each.)

fistulous opening was raw egg white (25 cc.), raw egg white (25 cc.) suspended in 200 cc. of distilled water and a mixture of raw egg white (25 cc.) desiccated ox bile (Difco 1 gm.) suspended in 100 cc. of distilled water. The accompanying graph gives the average gastric acid secreting response to these substances. Stomach contents (1 or 2 cc.) were removed at half hour intervals and titrated in the usual manner.

These experiments combined with those reported by Bateman[5] and by Arnold[4] tend to place raw egg white in a unique position as a protein food. The lack of gastric response and the increased permeability of the small intestine has caused us to investigate the possibility of the utilization of egg white and bile mixtures as vehicles for vaccines for oral administration. Human experiments are now in progress.

5077

Liver Autolysis in Vivo.

EDMUND ANDREWS AND LEO S. HRDINA.

From the Department of Surgery, University of Chicago.

The experimental work of Mason[1] called our attention to the extremely toxic reaction produced by the implantation of liver into the abdominal cavity of animals. Ellis and Dragstedt[2] showed that death of these animals was not strictly due to toxemia but there was a constant finding of large numbers of organisms of the *B. welchii* group in the abdominal cavity and drew attention to the fact that this *B. welchii* organism was a normal inhabitant of the livers of dogs. They implanted fetal liver into the abdomens of dogs and also adult livers which had been previously autoclaved and found that no toxic reaction ensued. Our experiments were undertaken with a view to establishing the mechanism of death in peritonitis due to liver autolysis. It was found earlier in the course of these experiments that if the liver was ground and allowed to autolyse and then sterilized that its implantation into the abdomen caused the same picture of an overwhelming *B. welchii* peritonitis as when the infected liver had been used. The seeming contradiction in the work of Ellis and Dragstedt is probably due to the fact that in their experiments the hard

1 Mason, E. C., *et al., J. Lab. and Clin. Med.*, 1924, x, 622.

2 Ellis, J. C., and Dragstedt, L. R., *Arch. Surg.*, 1930, xx, 8.

cooked mass of liver had less exposed surface and did not undergo autolysis so promptly. There is, therefore, some mechanism at work by which the ordinary flora of the intestine as represented by the Welch bacilli are allowed to make their way through the intestinal wall and infect these masses of autolysed liver which are shown to have been previously sterile by culture.

The following attempts were made to isolate the toxic factor in this reaction. 100 gm. specimens of liver were ground and incubated for 24 hours and then extracted with large amounts of water. They were boiled for several hours, filtered repeatedly and watery extracts concentrated down to 25 cc. These extracts were shown to be capable of producing the same typical autolytic peritonitis. Similarly extracts of liver which are ground and boiled promptly after removal have the same or almost the same toxic power.

In another series of experiments the livers were extracted repeatedly in large amounts of boiling water for several days followed by repeated extraction by boiling chloroform and alcohol and ether mixtures. The remaining substances, principally coagulated proteins, implanted within the abdomens of normal dogs were bringing about death within 24 hours from autolytic peritonitis.

The extracts of these livers were shown not to contain bile salts in sufficient amounts to produce any symptoms whatsoever, as shown by the work of Rewbridge recently reported here. These extracts give a strong reaction with Biuret test indicative of the presence of large amounts of peptones and proteoses. Extracts which have been subject to prolonged boiling in weak alkali solution no longer give the proteose violet Biuret reaction but only the red color of peptones and such extracts are no longer toxic. If to the strong solutions of these liver extracts chloroform be added there is a heavy precipitate of whitish material which can be separately removed in the separatory funnel, the chloroform extract evaporated in the water bath to dryness, and redissolved in water. This solution gives a strong Biuret reaction of the proteose type and the amount recovered from an extract of 100 gm. of liver will produce the autolytic peritonitis.

It is evident, therefore, that even the purified liver proteins on autolysis in the abdomen are capable of giving rise to this toxic factor. Extracts of autolysed liver were dialized from celloidin bags against large amounts of water and in this series of experiments the animals survived in most cases so that the toxin was dializable only with great difficulty.

New York Section.

5078

The Transformation of Pneumococcal Types in Vitro.

MARTIN H. DAWSON AND RICHARD H. P. SIA.*

From the Department of Medicine of the College of Physicians and Surgeons, and the Presbyterian Hospital, New York.

Griffith[1] was the first to show that type-specific, S, pneumococci may be transformed from one specific S type into other specific S types through the intermediate stage of the R form. He showed that the transformation of R forms, derived from one specific S type, into S forms of heterologous types may be effected *in vivo* by the following procedure: The subcutaneous injection, in white mice, of small amounts of living R forms together with suspensions of heterologous S cultures, killed by heating. Griffith further reported that all attempts to secure transformation of type by *in vitro* methods were unsuccessful.

Griffith's observations were confirmed and extended in recent publications by one of the authors.[2] In these communications it was also reported that all *in vitro* attempts to effect transformation of type were unsuccessful.

Recently we have renewed the *in vitro* studies and have succeeded in evolving a relatively simple technique for inducing transformation of pneumococcal types in the test tube.

For the purposes of convenience we have confined our present studies to the transformation of a 2R culture into Type III S organisms. However, since it has been shown in previous work that an R culture may be transformed *in vivo* into S organisms of *any* heterologous type it is probable that similar transformations may also be effected *in vitro*.

The procedure consists in seeding minimal amounts of an R culture into a suspension of S organisms of heterologous type, killed

* On leave of absence from the Peiping Union Medical College, Peiping, China.

1 Griffith, F., *J. Hygiene*, 1928, xxvii, 113.

2 Dawson M. H., *J. Exp. Med.*, 1930, li, 99, 123.

by heating. Certain conditions, while not absolutely necessary, apparently facilitate the transformation process. Some of these conditions are: (1) The amount of the R inoculum introduced, (2) the incubation of the cultures for a longer time than the conventional period, (3) the addition of a small amount (10%) of anti-R serum, (4) the addition of a small amount of blood-broth. The degree of heat to which the organisms have been exposed also materially affects the results. Suspensions of organisms heated for periods as long as four hours at 60°C. have been effective. Likewise suspensions of organisms heated for 15 minutes at 80°C. have been effective. However, organisms heated for 15 minutes at 100°C. have lost the capacity for inducing the transformation.

By this technique, transformation of type may be induced with very small quantities of heat-killed suspensions—quantities as small as the equivalent of 0.1 cc. of culture. Filtrates of actively growing cultures have not proven effective nor have filtrates of heat-killed suspensions of S organisms. Suspensions of S organisms, broken up by freezing and thawing and subsequently subjected to a temperature of 60°C. for 15 minutes have likewise proven ineffective. However, suspensions of S organisms first killed by heating for 15 minutes at 60°C. and subsequently frozen and thawed have proven highly effective. In this process the heat-killed cells, for the most part, maintain their integrity. In previous *in vivo* experiments autolysates of S cultures, heated for 15 minutes at 60°C., failed to induce the transformation. It would therefore appear that, under the conditions employed, the property of the heat-killed S organism responsible for bringing about change of type is destroyed or altered by the disruption of the S cell.

Experiments carried out under anaerobic conditions are now being undertaken.

In all experiments the vaccines were prepared in the same manner as described in previous publications. Similar controls were adopted to eliminate the possibility of the persistence of viable forms in the heat-killed suspensions.

5079

Studies on the Penetration of Dyes with the Glass Electrode. II. Penetration into Nitella from Solutions of Cresyl Blue, Azure B, and Methylene Blue Solution.

MARIAN IRWIN.

From the Laboratories of The Rockefeller Institute for Medical Research.

Two successive extractions of the sap were made from the vacuole of each living cell of *Nitella flexilis*. The first extraction, called for convenience "sap", was made without pressing on the cell wall, while the second extraction, called "sap mixture", was made by squeezing the cell wall vigorously. The pH value of the sap was found to be 0.6 pH lower than that of the sap mixture. This difference may be entirely due to the presence of the protoplasm in the latter, or partly due to the presence of fluid from the space between the cell wall and the protoplasm.

The pH values of the sap and the sap mixture remained unchanged during the period of extraction and of the subsequent measurement by the glass electrode.

The sap. (1) 0.07% cresyl blue, azure B, and methylene blue salt do not alter the pH value when dissolved in the sap *in vitro,* but 0.07% free base of cresyl blue and azure B cause it to increase about 0.5 pH with the former and 1 pH with the latter.

(2) *a.* When 0.07% dye has penetrated from the solution at pH 9.2 there is an increase in the pH value of the sap of 0.5 pH with cresyl blue, and 1 pH with the azure B or with the dye penetrating from methylene blue solution.

b. Spectrophotometric measurements show that cresyl blue and azure B penetrate as such while it is chiefly azure B that penetrates from methylene blue solution which always contains azure B as impurity.

(3) *a.* 0.07% cresyl blue penetrating in 15 minutes from the dye solution made up in buffer at pH 9.2 or at pH 6.8, or in tap water, raises the pH value of the sap equally (0.5 pH).

b. If cells are kept 15 minutes longer in the cresyl blue solution at pH 9.2, the dye continues to penetrate and raises the pH value still more, but if cells are transferred to the buffer solution at pH 9.2, containing no dye, there is no further penetration of dye and no further increase in the pH value of the sap.

The sap mixture. The sap mixture behaves toward these dyes like the sap when 0.07% dye salt or free base is added to it *in vitro.*

With penetration of cresyl blue there is practically no difference between these 2 extractions. But with the 0.07% dye penetrating from azure B and methylene blue solution at pH 9.2, there is only 0.6 pH increase in the sap mixture instead of 1 pH as in the case of sap. In the case of methylene blue, this difference is not due to the additional penetration of methylene blue salt into the protoplasm, because the dye in the sap mixture gives the same absorption curve as the dye in the sap, with the primary absorption maximum at 653 to 655 mμ (characteristic of a mixture of azure B and methylene blue, with the preponderance of the former).

It is concluded that cresyl blue penetrates chiefly as free base, and that azure B also penetrates chiefly as free base from azure B solution and from methylene blue solution at pH 9.2.

In these experiments contamination of the sap by the stained cell wall was avoided as previously described.

Impurity in the free base of dye when added to the sap *in vitro* does not alter the pH value.

5080

Studies on the Penetration of Dyes With the Glass Electrode. III. Penetration into Valonia of Cresyl Blue and Azure B.

MARIAN IRWIN.

From the Laboratories of The Rockefeller Institute for Medical Research.

(1) The pH value of the sap extracted from the vacuoles of small living cells of *Valonia macrophysa* is found to be pH 5.4. The pH value remains unaltered for several hours, *i. e.*, much longer than the period required for the extraction of sap and subsequent measurement with the glass electrode.

(2) 0.01% cresyl blue penetrating (in about 20 minutes) from the sea water at pH 9.5, raises the pH value of the sap 0.5 pH. This increase is 0.2 pH less than the rise brought about by the addition of 0.01% free base of cresyl blue to sap *in vitro*.

(3) 0.03% cresyl blue penetrating (in about 1 hour) raises the pH value about 0.9 pH, which is about 0.4 pH less than the rise brought about by the addition of 0.03% cresyl blue free base to sap *in vitro*.

(4) If cells are kept 8 hours longer in this cresyl blue solution, they appear to be still in good condition, so that it is likely that the cells described under (2) and (3) are not at all injured.

(5) No alteration in the pH value of the sap occurs when cells

are placed even for 8 hours in sea water at pH 9.5 containing no dye.

(6) Azure B penetrating the sap also increases the pH value, and this increase is again less than the increase brought about by addition of azure B free base *in vitro*. Owing to a slower penetration and greater toxicity of azure B as compared to cresyl blue, these results on azure B are rather unsatisfactory but experiments are being continued.

(7) The penetration of dye from methylene blue solution at pH 9.5 is still slower than from the azure B solution, so that sufficiently accurate results are not obtainable before the cells are injured but experiments are being continued.

It is concluded that cresyl blue and azure B free base penetrate the vacuoles of living cells of *Valonia macrophysa*.

The fact that the penetration of dye causes the pH value of the sap to increase less than when the same amount of free base of the dye is added *in vitro*, may in all probability be due partly to the production of acid by the cell rather than to the penetration of dye salt, just as was proved to be the case with the sap mixture of *Nitella*. It is partly due to the impurity in the free base of dye which slightly raises the pH value when added to the sap *in vitro*.

Owing to the fact that the sap of *Valonia* is not so well buffered as that of *Nitella* and also to the fact that the electrode is affected by the 0.6 M inorganic salt in the sap of *Valonia*, these results with *Valonia* are not so convincing as those with *Nitella*.

5081

Analysis of Ether-Air Mixtures by Thermal Conductivity Method.

F. H. HOWARD AND E. N. GOODMAN. (Introduced by E. L. Scott.)

From the Department of Physiology, College of Physicians and Surgeons.

For the purpose of rapid determination of ether concentration in ether-air mixtures we have found the thermal conductivity method of gas analysis satisfactory. Since the heat conductivity of ether vapor is only about one half as great as is that of air, the admixture of ether vapor with air decreases the loss of heat from the wire and causes its temperature to rise. The consequent increase of electrical resistance of the wire is readily determined and, if the apparatus is calibrated, the change of resistance is a measure of the ether concentration.

The apparatus used in this investigation consisted of a rectangu-

lar brass block 14x4x2 cm. in size through which 2 parallel holes 1 cm. in diameter were bored longitudinally. In each cell a platinum wire 0.005 cm. in diameter was suspended axially from plugs closing the ends, and heated by a current of 0.2 ampere. The gas was introduced through lateral tubes close to the ends of the cells. The calibration was effected by means of several ether-air mixtures of known concentration. These were made up by breaking, in a closed flask of known volume, a capillary tube containing a weighed quantity of ether. The mean experimental error of the determination was about 5% with concentrations between 0.3% and 1.3%. It is probably considerably less than this with mixtures of the usual anesthetic strength (6%-8%).

For concentrations of less than about 5% the resistance of the wire was approximately proportional to the concentration. When, however, larger concentrations were used proportionality no longer obtained. With concentrations greater than 10% the apparent heat conductivity began to increase until with mixtures of about 30% it was equal to that of the air. Mixtures stronger than 30% had a cooling effect greater than that of air. An explanation of the reversal of the apparent heat conductivity of mixtures of increasing concentration is to be found in the following facts. If a heated wire, 0.005 cm. in diameter, is suspended axially in a tube 1 cm. in diameter in a gas of about the density of air the loss of heat from the wire is entirely by conduction and convection currents do not occur. Langmuir[1] has shown that if the gas is more dense than air the stationary layer of gas surrounding the wire is less than 5 mm. in thickness. Hence to obviate entirely the development of convection currents in ether-air mixtures the radius of the tubular cell must be no greater than the thickness of the stationary layer. To test this theory a pair of cells 4 mm. in diameter was employed. We found that the resistance of the wire increased continuously, with increasing concentrations of ether up to 70%, beyond which observations were not made. There was no reversal of the apparent heat conductivity. From this we tentatively conclude that, in spite of the density of the gas mixture, the diameter of the cell was small enough largely to prevent convection currents.

While it is necessary to avoid cells whose diameter in proportion to the density of the gas would insure large convection currents and consequent unsteady bridge readings, the precision of the method is satisfactory when convection is reduced by proper design of the cell so that bridge readings are steady, whether or not convection is en-

[1] Langmuir, *Phys. Rev.*, 1912, xxxiv, 401.

tirely prevented. Since it is not easy to maintain the axial position of the wire in very narrow cells, it is better to use cells as large as possible without so greatly increasing the diameter as to secure troublesome convection currents. If the range of the concentrations to be measured is from 0 to 10% the 1 cm. cell with a wire of 0.005 cm. is satisfactory. For concentrations from 10% upward a cell of about 5 mm. diameter would be preferable.

As moist air has a heat conductivity different from that of dry air, it is necessary to dry the air in the reference cell and to dry the ether-air mixture as it is drawn into the cell. Calcium chloride has been found to be satisfactory for this purpose. It appears not to absorb any ether.

The temperature of the platinum wire carrying 0.2 ampere was calculated to be 68° above room temperature. There is good reason to believe that this temperature is certainly too low to cause ignition of the vapor, and in many hundreds of analyses with various percentages of ether no ignition has occurred. Since, however, the wire may break and a spark be formed, it is safer to disconnect the cell from the reservoir from which the specimen was drawn, before the current is turned on. If the cell is then left open at one end no harm would result from ignition.

5082

Further Note on the Relative Protection by Polymorphonuclear and Mononuclear Cells in Local Streptococcus Infection.

F. P. GAY AND A. R. CLARK.

From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University, New York.

Our investigations on the protective effect of granulation tissue (Gay and Morrison[1]) (Gay, Clark and Linton[2]) against a highly virulent streptococcus introduced into the pleural cavity of rabbits, led us to attribute a predominating if not exclusive rôle to mononuclears as compared with polymorphonuclear cells. In the acute stage of the inflammatory process when polymorphonuclears predominate the animals are fully as susceptible as normal controls. We have never denied that polymorphonuclears have a distinct protective energy with some bacteria and in some locations.

[1] Gay, F. P., and Morrison, L. F., *J. Am. Med. Assn.*, 1923, lxxx, 1298.

[2] Gay, F. P., Clark, A. R., and Linton, R. W., *Archiv. Path.*, 1926, l, 857.

Opie[3] in a recent article has reported similar experiments in the peritoneum and shown that early stages of inflammation (polymorphonuclear) prevent temporarily the invasion of the blood stream by the streptococcus, but further shows in complete confirmation of our results that recovery from such infection depends on the establishment of a later stage of inflammatory exudation. He does, however, suggest that our conclusions as to the negative or actually injurious effect of an excess of polymorphonuclear cells are open to another interpretation. It might be, to paraphrase and extend his remarks, that the sterile irritant (aleuronat) so long as it remains injurious is evidenced by polymorphonuclears and the introduction of a second irritant, the streptococcus, at this period leads to a fatal result. We have often thought of still another objection to our own conclusions, namely, that the pleural exudate in 20 hours is excessive in amount (5-10 cc. on the average) and might actually furnish a favorable location for the streptococcus to multiply. This particularly in view of our later observations that actual destruction of the streptococcus in the protected animals takes place not in the exudate itself but in the granulation tissue of the parietal pleura.

These considerations by Opie and by ourselves made it desirable to assure ourselves whether the accumulated polymorphonuclear cells in the pleura were in themselves endowed with any protective value. We have tried to determine the possible protective value of polymorphonuclear cells *per se,* unaffected by excessive exudation, in the following ways:

1. Aspiration of a large part of the 20 hour exudate in an irritated pleura before injecting the streptococcus does not result in protection.

2. Aspiration of exudate plus intravenous injection of antistreptococcus rabbit serum does not protect. Such addition of immune serum still further increases the protection afforded by granulation tissue (Gay and Clark[4]).

3. Infection of the opposite pleural cavity, where no histological change has followed aleuronat irritation of the other cavity, and where protection has been shown to occur by "transpleural mobilization of clasmatocytes" (Gay, Linton and Clark[5]) results in uniform fatality in the case of animals prepared only 24 hours previously.

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[3] Opie, E., *J. Immunol.,* 1929, xvii, 329.

[4] Gay, F. P., and Clark, A. R., *J. Exp. Med.,* in press.

[5] Gay, F. P., Linton, R. W., and Clark, A. R., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 23.

These observations make it still more convincing that polymorphonuclear cells are inactive in our experiments in which mononuclears have a marked protective effect.

5083

Sympathetic Activity After Prolonged Administration of Thyroxin.

NATHAN F. BLAU AND HELEN MC NAMARA.

From the Johnston Livingston Fund for Experimental Biochemistry, Department of Chemistry, Cornell University Medical College, New York, N. Y.

Innumerable "acute" experiments have been reported in the literature on the effect of thyroid administration on the pressor response of laboratory animals to adrenaline. The results have been in the main ambiguous. In contrast to these experiments, in this work an effort was made to insure a hyperthyroid condition of the animal by the long-continued administration of thyroxin.

Large vigorous cats were selected. Some were first fed Squibb's thyroxin tablets daily and then received daily intravenous injections of synthetic thyroxin (Hoffmann-La Roche). Others received the intravenous injections alone from the beginning of the period of thyroxin treatment. The total amount of thyroxin given per cat varied from 40 to 80 mg. The length of period of thyroxin administration varied from 35 to 57 days. At the end of that time blood pressure tracings were made from the carotid artery in the usual manner before and after the intravenous injection of a constant dose of adrenaline chloride (0.3 cc. per kg. of a 1:100,000 solution in saline). Paraldehyde (1.5 cc. per kg.) given by tube on an empty stomach was used as an anesthetic, as suggested by the work of Luckhardt and Koppanyi.[1] Sodium citrate (10% solution) served as an anticoagulant. Since some animals proved resistant to the action of thyroxin, only those were taken for the measurement of the pressor effect of adrenaline that showed a decided loss of body weight, 450 to 1000 gm. (1/3 to 1/7 of the original body weight). Five animals were finally chosen. Thirteen normal cats, treated exactly as those in the thyroxin series except for the administration of thyroxin, served as controls. Averages of the results for both series were as follows:

[1] Luckhardt, A. B., and Koppanyi, T., *Am. J. Physiol.*, 1927, lxxxi, 436.

TABLE I.

	Normal	Hyperthyroid
Average systolic blood pressure range before adrenaline	mm. 134-149	mm. 123-151
Average systolic blood pressure range after adrenaline	157-176	170-187
Average rise in systolic blood pressure after adrenaline	24	46
Average percentage rise in systolic blood pressure after adrenaline	17%	35%

The data clearly show that the same dose of adrenaline produces in the hyperthyroid cat a rise in blood pressure averaging 106% higher than in the normal control animals. Since no alkaline solution of thyroxin was injected into the animals during the measurement of the blood pressure and no artificial respiration was used, there is no reason for assuming that the greater vascular response was due to any increase in the pH of the blood. Neither could we observe any gradual increase of excitability of the autonomic nerves accompanying successive intravenous injections of constant doses of adrenaline as was reported by Lieb and Hyman[2] for pithed, decerebrated cats maintained by artificial respiration.

A striking result of the faradic stimulation of the peripheral end of the divided splanchnic nerve was a marked dilatation of the pupils of the thyroxin-treated cats. In normal cats splanchnic stimulation produced no such reaction, in agreement with the results of Stewart, Rogoff and Gibson.[3] The hemodynamic response following splanchnic stimulation in thyroxin-treated animals was not markedly different from that shown by the normal controls. The insufficient number of observations on this point, however, does not permit of any definite conclusion.

Electrocardiographic studies before and after the administration of thyroxin seem to show definite changes in the cardiac response of hyperthyroid cats to adrenaline. These will be presented in a later report. Work is still in progress and is being extended to several phases of thyroid-adrenal inter-relationship.

[2] Lieb, C. C., and Hyman, H. T., *Am. J. Physiol.*, 1922-23, lxiii, 60.

[3] Stewart, G. N., Rogoff, J. M., and Gibson, F. S., *J. Pharm. and Exp. Therap.*, 1916, viii, 205.

5084

Increased Resistance of Rachitic Rats Fed Irradiated Food.

ELIZABETH CHANT ROBERTSON AND JOHN R. ROSS.
(Introduced by F. F. Tisdall.)

From the Research Laboratories of the Sub-department of Pediatrics, University of Toronto, and the Hospital for Sick Children, Toronto, under the direction of Alan Brown, M. B.

A number of albino rats 24 to 27 days old were separated into 2 groups, whenever possible by dividing litters, each week. The diet of the breeding rats had previously been regulated so that there was no excess of vitamin D. Group 1 was fed a modification of McCollum's[1] rachitogenic diet 3143 in which the usual 33% of whole wheat was replaced by 33% of non-irradiated muffets, which are made of whole wheat. The rats of this group were usually slightly heavier (1 to 2 gm.) at the outset than the rats in group 2. Group 2 was fed a diet identical in every respect except that the muffets had been irradiated. After 3 weeks on this diet the average weight of the group 1 rats (non-irradiated food) was 44 gm., and of the group 2 rats (irradiated food) 46 gm. In an individual experiment the variations in weight in the two groups were often the same. After the 3 weeks on the diet the rats were starved for 6 hours, then

TABLE I.

	Group 1 (Non-irradiated Muffets)				Group 2 (Irradiated Muffets)		
Exp.	Infecting Dose of Broth Culture	No. of Rats	No. of Survivors	Ave. lgth. of life of rats dying	No. of Rats	No. of Survivors	Ave. lgth. of life of rats dying
	cc.			days			days
1	0.5	3	0	8.3	2	2	—
2	0.5	7	0	7.6	4	3	5.0
3	0.4	3	0	7.0	1	1	—
4	0.5	6	0	9.1	8	2	8.5
5	0.1-0.3	14	0	7.2	14	2	9.3
6*	0.4	6	0	7.0	9	3	12.0
7*	0.6	10	0	9.5	10	1	12.5
8*	0.4	5	0	8.4	4	1	7.0
9	0.1	6	1	9.0	7	2	8.4
10	0.05	7	2	7.6	13	8	19.2
11	0.05	10	0	10.9	13	2	10.2
12	0.05	12	1	8.9	9	1	13.5
Total		89	4	8.6	94	28	11.1

* Were given 30% muffets and 6% yeast.

[1] McCollum, E. V., Simmonds, N., Shipley, P. G., and Park, E. A., *J. Biol. Chem.*, 1921, xlvii, 507.

weighed and put in individual cages. Every rat in a single experiment was then fed the same small amount (see Table I, column 2) of an 18 hours' broth culture of *Salmonella muriotitis* on a small piece of dried bread. After the bread was eaten the previous diet was restored. This salmonella strain which was isolated from albino rats by McCordock,[2] can not be differentiated from *S. enteriditis (B. enteriditis)* by its biological reactions, but serologically it appears to be distinct. *S. enteriditis* is known to cause epidemics of severe and often fatal diarrhea in rats. The rats, therefore, were being infected with a rat disease by the most natural route *(per os)*. The stock strain was subcultured weekly on plain agar slants and there was no evidence of decreasing virulence.

Death usually took place in 6 to 10 days, although occasionally a rat lived as long as 21 days. Cyanosis, blood stained discharge from the nose and eyes, weakness and diarrhea usually accompany the infection and convulsions frequently precede death. Of the rats which died, the group 1 rats (non-irradiated food) usually lived a shorter time than the group 2 rats (irradiated food), see Table I, columns 5 and 8. Post mortem examinations were made on all the dead rats and cultures were always made from the heart's blood and occasionally from the spleen. Cultures were identified as *S. muriotitis* if they were Lactose—, Dextrose+, and Saccharose— and if they were agglutinated in dilutions of 1:1000 or over with *S. muriotitis* immune serum.

Of 85 non-irradiated food rats which died, 80 yielded cultures of *S. muriotitis* from their heart's blood (mostly pure). Of the 66 irradiated food rats which died, 56 showed similar cultures. All spleen cultures yielded *S. muriotitis*. Practically all of the dead showed blood in the pyloric portion of the stomach and in the small intestine. Macroscopic ulcers were seen in the pyloric part of the stomach of 5 rats. Necrotic and hemorrhagic areas in the gastric and intestinal mucosa and in the liver were shown on microscopic section.

Survivors. Twenty-eight of the rats fed the irradiated food (group 2) withstood the infection as compared with 4 fed the non-irradiated food (group 1), see Table I, columns 7 and 4. The survivors were allowed to live 28 days, when they were killed with ether and examined. All of these heart's blood cultures were sterile.

Controls. As *S. enteriditis* is not infrequently found in normal rats, blood cultures were made on a series of rachitic rats. These

[2] McCordock, H. A., *Bull. Johns Hop. Hosp.*, 1925, xxxvii, 412.

animals were kept in a separate animal room and had no contact with our infected rats. Twelve blood cultures have been made up to the present and these have all been sterile. More of these controls will be examined.

Fecal cultures were made on 47 of the rats used in the resistance experiments the day before they were infected, and only 3 of these showed *S. muriotitis*. Consequently it is thought that the recovery of *S. muriotitis* from the blood of a dead rat, after it has been fed this organism suggests very strongly that the rat died of a muriotitis infection and it is not likely that the disease originated from the rat itself.

Two typical rats from each group were set aside for chemical controls, and were fed a small amount of sterile broth, instead of the culture, on a similar piece of bread. After a week in the individual cages, that is after 4 weeks of the diet, the whole blood phosphorus was determined by the method described by Tisdall[3] and the bone ash percentage by that of Bethke, Steenbock, and Nelson.[4] The results are shown in Table II.

TABLE II.

Group 1 (Non-irradiated Muffets)				Group 2 (Irradiated Muffets)		
Exp.	Blood Phosphorus mgm. per 100 cc.	Bone Ash %	X-ray Showing Rickets	Blood Phosphorus mgm. per 100 cc.	Bone Ash %	X-ray Showing
4	—	—	—	1.5	41.1	—
5	2.5	31.9	marked	3.7	42.6	no rickets
6	2.5	37.3	"	3.6	46.1	" "
7	1.4	37.5	moderate	3.1	49.3	" "
8	—	36.0	"	—	—	—
9	2.5	31.7	marked	2.4	42.2	slight rickets
10	1.4	39.8	"	3.2	47.9	no rickets
11	3.1	27.7	"	3.6	40.0	" "
12	2.8	37.4	moderate	3.5	48.8	slight rickets

The resistance-raising properties of cod liver oil, irradiated ergosterol and sunlight through vita glass are being investigated. Direct sunshine has been previously shown[5] to raise the resistance of rachitic rats.

Summary. Of 89 rats fed a rachitic diet including non-irradiated muffets, 5% survived a *per os* enteriditis infection, as compared with 30% of 94 rats fed the same diet including irradiated muf-

[3] Tisdall, F. F., *J. Biol. Chem.*, 1922, l, 329.

[4] Bethke, R. M., Steenbock, H., and Nelson, M. T., *J. Biol. Chem.*, 1923, lviii, 71.

[5] Robertson, E. C., *Am. J. Hyg.*, 1929, ix, 75.

fets. Therefore we conclude that the use of irradiated whole wheat (muffets) in a rachitogenic diet fed to rats increases their resistance against infection.

5085

Experimental Edema—Further Observations on the Plasma Proteins and Blood Cholesterol.

LOUIS LEITER.

From the Lasker Foundation for Medical Research and the Department of Medicine, University of Chicago.

Since the first report, in 1928,[1] of the experimental production of edema in dogs by the method of plasmapheresis, further work has been carried out, the results of which are given in this preliminary report.

Edema has been produced in about 40 dogs, often several times in the same dog, whenever the plasma protein level has been maintained at about 3 gm. % or below. Above this level, edema disappeared promptly. There has been essentially no exception among the dogs used.

Several factors have been controlled. *Cardiac damage* has been ruled out by bleeding a series of dogs from the external jugular veins. *Starvation* alone, or with the daily administration of 1500 cc. of saline by stomach tube, gives rise to no significant change in the concentration of the plasma proteins nor to visible edema. *The alkalinity of the modified Locke's solution* used as a suspending medium for the erythrocytes injected into the dog plays no important rôle.

The *regeneration* of the *plasma globulin is much more rapid than that of the plasma albumin* and accounts for most of the sharp rise in the total protein when plasmapheresis is discontinued. This is shown best in the long experiments in which the plasma albumin level remains at about 1 or 1½% with relatively slight fluctuations up or down, while the plasma globulin curve shows striking variations depending upon whether or not plasma depletion is being carried out. The total protein curve parallels closely that for globulin. These results confirm the earlier work of Kerr, Hurwitz and Whipple.[2] It is easy to see, therefore, why the albumin/globulin ratio is

[1] Leiter, L., PROC. SOC. EXP. BIOL. AND MED., 1928, xxvi, 173.

[2] Kerr, W. J., Hurwitz, J. H., and Whipple, G. H., *Am. J. Physiol.*, 1918, xlvii, 356.

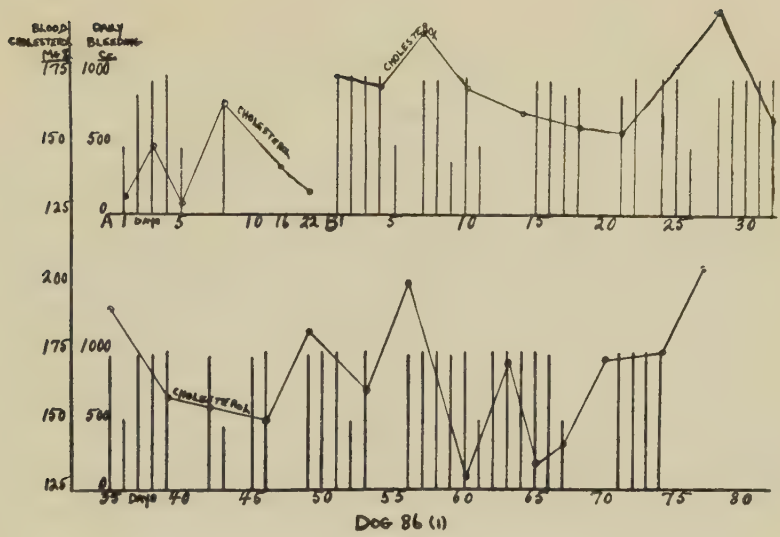

CHART 1.

The *blood cholesterol curve during prolonged plasmapheresis.* The vertical lines indicate the volume of daily plasmapheresis. *Note the tendency for the blood cholesterol to rise in the intervals between active plasmapheresis.*

usually reversed when there has been a long continued loss of plasma protein.

The *cholesterol level of the blood* is quite variable in the experiments of less than 2 or 3 weeks' duration. However, in the longer experiments (Chart 1), there seems to be a fairly definite cycle in the sense that the *cholesterol falls after several days of vigorous plasma depletion* but *rises sharply on cessation of plasmapheresis for a few days.* There is no persistent increase in the blood cholesterol. In this respect the clinical nephrotic syndrome presents the opposite picture.

These experiments bear no relation to the clinical nephrotic syndrome other than that they confirm the causal connection between low plasma proteins and a low protein, filtration type of edema fluid, which does not require the assumption of cardiac, renal, vascular or tissue colloid disturbance for its existence.

5086

Effects of Certain Light and Soil Characteristics on the Balance of Mineral Nutrients in Triticum.

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From the Plant Physiology Laboratory, State University of Iowa.

In earlier investigations it was observed that foliar chlorosis developed during periods of intense insolation of grain plants grown on humus soils which had been limed to correct acidity. In order to determine the cause of this injury 2 sets of Marquis wheat were grown, one on strongly acid humus soil low in mineral matter and the other on a fertile but acid loam. The acidity of a portion of the soil in each set was corrected by applications of pulverized calcium carbonate. A white muslin screen was installed over one-half of the plants when 6 weeks old, the remainder being left exposed to full illumination.

After symptoms of chlorosis had developed in fully insolated plants, entire tops were cut from several plants in each set at 4-hour intervals, frozen, comminuted and pressed under uniform pressure. The expressed sap was collected and its hydrion concentration measured potentiometrically by means of the calomel half cells and a quinhydrone electrode.

The data indicate that chlorosis was definitely correlated with sap hydrion changes attributable to differences in soil reaction and light intensity. The acidity of sap expressed at 4-hour intervals disclosed a diurnal periodicity in all plants but most pronounced in the plants on limed soil under full insolation. Reversals in the acidity gradient were not conincident with the hour of maximal light intensity, which suggests that the thermal factor in insolation may not be as important as its light effect.

Light intensity and soil alkalinity exercised considerable influence upon the level of acidity attained. Limed cultures in the shaded and unshaded sets maintained a lower degree of acidity and exhibited a greater initial decrease in sap hydrion concentration than the corresponding untreated plants exposed to full light intensity. The pH values of the sap from the limed cultures exposed to strong insolation for several consecutive days show that the period of darkness is too brief for complete acid recovery. Hence acidity falls progressively to lower levels and shows smaller diurnal fluctuations during prolonged periods of strong illumination. Microchemical analyses of leaves show that the diminished acidity of the

sap increasingly interferes with iron mobility and finally induces chlorosis such as became apparent in the 8-week-old plants on the limed humus.

The onset of chlorosis in young leaves was delayed and was less severe in plants which were shaded following the use of lime. This fact together with the pH values of these plants, suggests that shading to a considerable extent offsets the iron insufficiency created by lime. The acid recovery of limed plants in shade does not, however, reach the original level of acidity found in the sap of plants grown on the untreated humus. Microchemical inspection of chlorotic plants disclosed an abundance of iron in the roots but little or none in young leaves.

The most vigorous plants grew on the loam soil and the poorest on the fully illuminated, untreated and limed humus. Treated and untreated plants in this soil, though both low in vigor and retarded in development, represent opposite extremes of sap acidity. Seeds in the untreated humus germinated rapidly and the seedlings to all outward appearances grew normally until the fifth week. The rate of stem elongation then diminished and the leaves remained narrow, though increasing in length. During the eighth week the older leaves turned a dull green while their tips became flaccid and turned brown. The leaves then gradually died back toward the stem. The foregoing symptoms differed from those displayed by plants on the same soil after it had been limed. In the latter instance new leaves became noticeably chlorotic during the eighth week, the severity of this condition increasing with age. Reduced size and delay in maturation marked both the limed and untreated plants on the humus soil. The hyperacidity of the plant on untreated humus and the alkalinity of the strongly insolated, limed plants appeared equally injurious to wheat in its formative stages.

During the tenth week, it was found that mature leaves of fully illuminated, chlorotic plants on the limed humus gradually began to lose their turgor and turn brown. Many of the oldest leaves wilted and died. Acidity tests made on these plants during the incipient stages of browning disclosed that the sap hydrion concentration had risen considerably above that found in the 8-week-old plants of the same series. Moisture content had also fallen about 5%. Diurnal acid periodicity could no longer be detected and the hydrion level approximated that of plants growing on the untreated acid humus. This latent increase in the acidity of initially low-acid chlorotic plants is due to the accumulation of acid catabolic products and to the decrease in tissue fluids. The extremes of high and low acid-

ity both eventually result in sap hyperacidity. The untreated loam produced more vigorous plants than the untreated, low-mineral humus though both soils were strongly acid. The higher mineral content of the acid loam may in part account for the better growth of wheat on this soil.

Fluctuations in light intensity and soil acidity produced smaller changes in sap hydrogen concentration of plants grown on the loam soil. Under certain conditions, such as lack of balance among nutrients of mineral insufficiency, it seems that the effect of lime in altering the free acidity of the sap may outweigh its other functions as a nutrient.

5087

Blood Findings in Albino Rats Suffering From Lack of Vitamin A.

R. G. TURNER. (Introduced by E. W. Rockwood.)

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The literature regarding blood changes in vitamin A deficiency disease deals chiefly with blood counts, platelets and hemoglobin findings. Cramer, Drew and Mottram[1] in a study of blood platelets and corpuscle counts stated that there were no constant differences in the number of white or red corpuscles, although in advanced stages of deficiency there may be a distinct anemia. Koessler, Mauer and Loughlin[2] report that they have produced conditions similar to human pernicious anemia in experimental animals deprived of vitamin A. Hopp[3] studied the occurrence of anemia in rats on deficient diets and concluded that diets deficient in vitamins A and B do not produce anemia in the rat.

Damianovich and collaborators[4] did not observe an anemia in rats suffering from lack of A or B. Falconer[5] reports a slight drop in platelets and a small rise in red and white corpuscles, but con-

[1] Cramer, W., Drew, A. H., and Mottram, J. C., *Proc. Royal Soc.*, London, Series B, 1922, xciii, 499.

[2] Koessler, Karl K., Maurer, S., and Loughlin, R., *J. Am. Med. Assn.*, 1926, xxcvii, 476.

[3] Hopp, W. H., *Johns Hopkins Bull.*, 1922, xxxiii, 163.

[4] Damianovich, H., Bianchi, A., and Savazzini, Lilia A., *Compt. Rend. Soc. de Biol.*, 1923, xxcviii, 377.

[5] Falconer, E. H., and Peachy, G., *Am. J. Physiol.*, 1926, lxxvi, 145.

cludes the changes are not sufficient to constitute specific lesions of vitamin A deficiency. Recently Anderson and his co-workers[6] published values on the composition of normal rat blood. They find that in most respects it is quite similar in composition to human blood. The most marked exception is a higher value for non-protein nitrogen.

This study was to determine whether there is any decided change in the relative number of red blood corpuscles or the amount of hemoglobin, uric acid, non-protein nitrogen, creatinine, urea nitrogen, chlorides and sugar in the blood of xerophthalmic animals as compared to that of normal stock rats and control animals. The latter received cod liver oil in addition to the vitamin A-free diet.[7] The animals were placed on diet at varying ages. They were not all obtained from the same stock colony which accounts for the variation in the length of time required for development of xerophthalmia. To determine the amounts of these blood constituents in the rat it was necessary to take the combined blood of 4 animals. The blood for chemical examination was obtained as follows: The animal was first placed in a state of complete surgical anesthesia. The hair was removed from the under side of the neck, and the carotid artery exposed and severed. By holding the animal in the hand the blood was poured into an oxylated test tube which was held at the opening in the neck. The findings of the determinations in each group of the combined bloods tested were averaged and taken for the standard of that group. The hemoglobin and red cell counts given were taken for each individual rat making a total of 28 stock, 58 xerophthalmic, and 13 control animals.

The combined bloods of each 4 rats were deproteinized by the method of Folin and Wu.[8] Uric acid was determined by the method of Benedict,[9] non-protein nitrogen and creatinine by the method of Folin and Wu.[8] Urea nitrogen was estimated by Folin's method, using the aeration process.[10] Chlorides were determined by Whitehorn's method.[11] Sugar values were obtained by the method of Folin and Wu.[12] The acid hematin method of Newcomer[13] was

[6] Anderson, A. K., Honeywell, H. E., Santy, A. C., and Pedersen, S., *J. Biol. Chem.*, 1930, lxxxvi, 157.

[7] Turner, R. G., PROC. SOC. EXP. BIOL. AND MED., 1928, xxvi, 23.

[8] Folin, O., and Wu, H., *J. Biol. Chem.*, 1919, xxxviii, 81.

[9] Benedict, S. R., *J. Biol. Chem.*, 1922, liv, 233.

[10] Hawk, P. B., and Bergeim, O., "Practical Physiological Chemistry," 1927, P. Blakiston's Son & Co., 371.

[11] Whitehorn, J. C., *J. Biol. Chem.*, 1920-21, xlv, 449.

[12] Folin, O., and Wu, H., *J. Biol. Chem.*, 1920, xli, 367.

[13] Newcomer, H. S., *J. Biol. Chem.*, 1919, xxxvii, 465; 1923, lv, 569.

used for estimating hemoglobin. Red cell counts were made by the Levy-Burker-Neubauer modification of the Thoma method.[14]

The blood obtained for hemoglobin and red blood cell counts was taken from the tail of the rat. The end of the tail was clipped and the blood allowed to flow freely before taken for the tests.

All the tests on animals suffering from lack of vitamin A were made at varying times after symptoms of definite xerophthalmia appeared.

The results are given in the following tables. Table I gives the average blood chemistry findings for each group, Table II, hemoglobin and red blood cell counts as found in the blood of normal stock, xerophthalmic, and control animals.

As seen in Table I for all 3 groups, uric acid, creatinine, sugar, and chloride values are similar to those given by Anderson for normal rat blood. They all fall, except the latter, within the range for normal human blood. Chloride values are slightly below normal. Non-protein nitrogen averages below the range for normal rat blood in stock and control animals. The xerophthalmic animals show an average within the range as compared with his findings. Two of the 7 determinations in this group gave values strikingly above the normal

TABLE I.
Average Blood Findings in Normal, Xerophthalmic and Control Animals.
Results in mgm. per 100 cc. of blood.

No. of Animals	Uric Acid	Non-Protein N.	Creatinine	Urea N.	Chloride as NaCl	Sugar
Normal Stock Animals.						
12						
Range	2.6-3.1	30.9-31.9	1.5-1.6	15.0-16.0	330-410	100-129
Ave.	2.9	31.5	1.5	15.4	400	115
Xerophthalmic Animals.						
36						
Range	1.6-3.0	35.4-58.8	1.4-1.9	15.2-28.6	320-440	97-130
Ave.	2.5	40.1	1.5	19.8	390	112
Control Animals.						
16						
Range	2.5-3.8	36.0-37.2	1.5-1.6	16.0-18.2	270-630	95-129
Ave.	2.5	36.5	1.55	16.7	440	111
Normal Rat Blood (6)						
Range	1.35-2.4	38.6-49.1	1.2-1.4	9.9-20.6	472-572	110-132
Ave.	1.86	45.2	1.28	15.6	515.2	122.2

[14] Morris, R., "Chem. Laboratory Diagnosis," Appleton & Co., 1923.

TABLE II.
Hemoglobin and Red Blood Cell Count in Normal, Xerophthalmic, and Control Animals.

No. of Animals	Age in Days	Body Weight in gm. Aver.	Sex	Hb gm. per 100 cc. Aver.	Deviation	R.B.C. Million per cmm. Aver.	No of Days on Diet
Normal Stock Animals.							
8	80	114	♂	16.64	±3	8.72	59
4	78±2	90	♀	15.65	±1	8.20	57
16	65±4	76	♀	12.94	±2	8.18	44
Aver. Unweigt.	74	93		15.07		8.37	53
Xerophthalmic Animals.							
29	98±2	119	♀	13.58	±3	9.16	54
8	80	94	♀	14.99	±1	8.30	38
2	70±4	52	♂	15.49	—	10.06	45
19	200±5	110	♀	13.51	±2	8.24	130
Aver. Unweigt.	112	93		14.39		8.94	66
Control Animals.							
13	223±5	140	♀	13.31	±2	8.39	153

(48.7 and 58.8). Figures corresponding to these high values were found by Anderson in rats with bad lungs. His animals ranged from 7 to 12 months in age. Our animals at necropsy showed no macroscopical lung lesions. They ranged from 2 to 7 months in age.

The urea nitrogen findings show an average of 15.4 mg. per 100 cc. blood for normal stock animals, 19.8 for xerophthalmic, and 16.7 for control animals. The basal ration for xerophthalmic and control animals was lower in nitrogen content than the stock ration. The greater increase as found for the diseased animals may be due to the emaciated condition of these animals because of excess nitrogen formed through destruction of protein tissue.

The hemoglobin determinations given in Table II do not show an increase in xerophthalmic animals.

The red blood cell counts show an average increase of 560,000 cells in the xerophthalmic animals as compared with stock and control animals.

Summary. From the results given it is concluded that the relative amounts of uric acid, non-protein nitrogen, creatinine, urea nitrogen, chlorides and sugar in the blood of xerophthalmic animals are not striking enough or constant enough to constitute a decided

change. Further, the hemoglobin and red cell count are not sufficiently altered to show specific lesions in vitamin A deficient animals.

It is believed that anemia does not result from lack of vitamin A.

5088

A Test to Differentiate Irradiated Cholesterol From Non-Irradiated Cholesterol and Irradiated Ergosterol From Non-Irradiated Ergosterol.

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Cholesterol dissolved in chloroform gives with a mixture of sulphuric and selenious acids (125 mg. of sodium selenite in 25 cc. of concentrated sulphuric acid) a purple color in the upper chloroform layer and a red-brown color in the lower acid layer, which displays no green fluorescence.[1] Solid cholesterol, irradiated with the mercury arc lamp for 30 minutes at a distance of 16 inches, when dissolved in chloroform gives with the sulphuric and selenious acid mixture a dark wine-red color in the chloroform layer, and a still darker wine-red color in the acid layer with the absence of green fluorescence. This reaction takes place in the 1.0% and in the 0.5% solution. In the 0.25%, 0.10%, 0.025% and 0.01% solutions the color reaction is negative. The chloroform layer is more or less purple, while the acid layer is brown and free from fluorescence.

Ergosterol, non-irradiated and dissolved in chloroform, gives with sulphuric acid a colorless chloroform layer and a brownish red, cherry red or orange color in the acid layer in direct light and a green fluorescence in transmitted light.[2] With the sulphuric acid-selenious acid mixture the chloroform layer also remains uncolored, while the acid layer gives a brown color in direct light and no green fluorescence in transmitted light.

Solid ergosterol, irradiated with the mercury lamp for 30 minutes at a distance of 16 inches, dissolved in chloroform and treated with

[1] Levine, V. E., and Richman, E., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 832.

[2] Levine, V. E., and Richman, E., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 833.

sulphuric or sulphuric-selenious acid, gives a greenish blue color in the chloroform layer in the 1.0% and in the 0.5% solution. The acid layer, however, does not differ in color from that obtained with non-irradiated ergosterol. In the 0.25%, 0.1%, 0.025% and 0.01% solutions of irradiated ergosterol the color responses were the same as those obtained with the same concentrations of non-irradiated ergosterol.

Irradiated cholesterol and irradiated ergosterol give in 1.0% and 0.5% chloroform solutions tests different from those obtained with the same sterols non-irradiated. Lower concentrations of the irradiated sterols give the same tests as the non-irradiated substances. These facts argue for the possibility that irradiation has produced a new compound which is present in small quantities in the higher concentrations, but which is absent altogether or present in too low a dilution to give the test in the lower concentrations.

5089

Stimulating Influence of the Anterior Pituitary Upon the Squamous Epithelium of the Cervix Uteri.

J. HOFBAUER. (Introduced by E. M. K. Geiling.)

From the Department of Obstetrics, Johns Hopkins University and Hospital.

The immediate and later results upon the ovaries and the uterus of the implantation of anterior pituitary substance, or of the injection of its growth-hormone into rodents (rat, mouse), as well as in dogs, has of late been extensively described in the literature. The acceleration of both the maturation of follicles and the formation of corpora lutea, as well as the hypertrophy of the uterine musculature under these conditions have recently been established by important experimental studies. (Evans,[1] Smith and Engle,[2] Zondek and Aschheim,[3] Putnam[4]) All of these investigators agree concerning the stimulating effect of the hormonic principle on the tissues enumerated.

In the present study evidence has been obtained indicating that an overgrowth of the squamous epithelium covering the vaginal

[1] Evans, H. M., *Harvey Lectures*, 1924, xix, 212.

[2] Smith, P. E., and Engle, E. T., *Am. J. Anat.*, 1927, xl, 173.

[3] Zondek, B., and Aschheim, S., *Klin. Wochensch.*, 1927, vi, 323.

[4] Putnam, S., *Archiv. Surg.*, 1929, xviii, 1708; *Am. J. Med. Sciences*, 1930, lii, 244.

portion of the uterus can be initiated by means of either repeated intraperitoneal administration of extracts or by intramuscular transplantation of bits of the anterior lobe of the beef. Adult guinea pigs have been used in these experiments exclusively. In one series of our experiments, double ovariectomy was performed 2 weeks prior to the administration of anterior pituitary extract, for whose preparation I am under many obligations to Dr. E. M. K. Geiling.

The proliferation of the squamous epithelium on the outer surface of the cervix is shown by the development of epithelial prolongations, which in places extend deep into the connective tissue; but no epithelial pearls are found in these areas. Occasionally, the invading epithelial columns are fronted by a slight small cell infiltration. Mitotic figures are scarce. The histologic appearance is very characteristic. The ablation of the ovaries appears to promote this process of proliferation, as it is more pronounced than when the ovaries are retained.

According to the generally prevailing nomenclature it seems correct to designate the condition as *leucoplakia,* and the researches of Pemberton,[5] Hinselmann[6] and v. Franqué[7] indicate that benign leucoplakia is to be regarded as a precancerous condition. An additional point demands special consideration. Recent studies on the development of the upper part of the vagina and of the cervix in human foetuses, by Koff[8] in the Carnegie Institute of Embryology, reveal the fact that at a certain period of development (126-150 mm. in length) a sudden phase of activity occurs in the vaginal cord, when areas of proliferating epithelium develop, which form excrescences from the original smooth wall. In some areas the growth is invading the stroma so actively that at first sight it appears almost malignant. It would, accordingly, appear that an almost complete analogy exists between a certain phase of the embryonic development of the cervix on one hand, and that noted in our observations on the other. In other words, the experimental data here recorded find their counterpart in a certain phase of intrauterine life. This would indicate that in the adult the squamous epithelium covering the vaginal portion of the cervix retains its primordial character and under the influence of certain stimuli may resume its embryonic type and potentialities.

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[5] Pemberton, F. A., *Am. J. Obst. and Gynecol.,* 1929, xvii, 126.

[6] Hinselmann, H., *Z. f. Geb. und Gyn.,* 1930, xcvii, 216.

[7] Franqué, O., *Centralbl. f. Gynaec.,* 1927, lvi, 822.

[8] Koff, A., to be published in *Contributions to Embryology* and *Reports of the Carnegie Institute,* Washington.

Possibly further studies will show that the response of cervical epithelium to the pituitary stimulus is due to its being a derivative of the coelomic epithelium.

5090

Hypoglycemia in Protracted Anaphylactic and Tuberculin Shock.

L. DIENES.

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When guinea pigs die in protracted anaphylactic shock,[1] death is usually preceded by a comatose condition with intermittent forced breathing and occasional convulsions. Sometimes this condition, in which the animals are insensitive to pain, lasts for several hours. These cases are suggestive that the comatose condition is produced by factors which are not present in the milder forms of the anaphylactic intoxication or in those cases where death occurs earlier after injection. The examination of the sugar content of the blood seemed indicated as the liver plays an important rôle in the production of protracted anaphylactic shock, also it is known that anaphylactic shock has a pronounced influence on the sugar content of the blood.[2] In addition in different bacterial intoxications hypoglycemia was observed.[3]

TABLE I.
Sugar Content of the Blood of Guinea Pigs Dying in Protracted Anaphylactic and Tuberculin Shock.

Guinea Pig		Interval Between Injection and Death. Hours	Sugar in the Blood
331	Protracted Anaphylactic Shock	3	.28
334		3	.22
373		2	.20
476		3¾	.03
473		6	.035
412		9	.03
156	Tuberculin Shock	3	.15
157		6½	.095
187		22	.08
484		4½	.025
486		5	.03
127		8	.03

1 Dienes, L., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 690.

2 Zeckwer, I. T., and Nadler, J. E., *J. Exp. Med.*, 1929, xlix, 481.

3 Menten, M. L., and Kipp, H. A., *J. Infect. Dis.*, 1930, xlvi, 267.

The data given in the table are typical. Death, both in protracted anaphylactic shock and in tuberculin shock might occur with high, normal, or very low sugar values in the blood. In anaphylactic shock during the first 2-3 hours after the injection (also when the guinea pig dies in acute shock, or survives) the sugar content of the blood is increased. Later in the severe or fatal shock we find very low sugar values. This regularity was not as pronounced in tuberculin shock, probably because the stage and extent of the disease influences the reaction of the animal.

It is probable that the excessive hypoglycemia plays a rôle in the death of the animal. The occurrence of death both with high and with very low sugar values indicates that death is caused by different combinations of factors, both in protracted anaphylactic shock and in tuberculin shock.

The sugar determinations were made with the new method of Folin and Wu,[4] and in a number of cases we made parallel determinations, using the method of Hagedorn and Jensen.[5] The blood was taken from the heart before the death of the animal.

5091

Ergotoxine Miosis.

F. F. YONKMAN. (Introduced by Sanford B. Hooker.)

From the Department of Pharmacology, Boston University, and Evans Memorial Hospital.

In 1906 Dale[1] demonstrated constriction of the cat's iris by ergotoxine and attributed this to direct stimulation of the iris sphincter. Recently Koppanyi[2] reported that a sufficient concentration of ergotamine injected intraocularly paralyzes the motor sympathetic terminations in the dilatator pupillae and stated that the abolition of sympathetic tonus was sufficient basis for explanation of ergotoxine constriction.

Our results of numerous studies on the isolated sphincter muscle of the iris in an oxygenated Locke-Ringer bath warrant the support of Dale's conclusion that ergotoxine constricts this muscle to a con-

[4] Folin, O., *J. Biol. Chem.*, 1929, lxxxii, 83.

[5] Hagedorn, H. C., and Jensen, B. W., *Biochemische Z.*, 1923, cxxxv, 46.

[1] Dale, H. H., *J. Physiol.*, 1906, xxxiv, 163.

[2] Koppanyi, T., *J. Pharm. and Exp. Therap.*, 1930, xxxviii, 101.

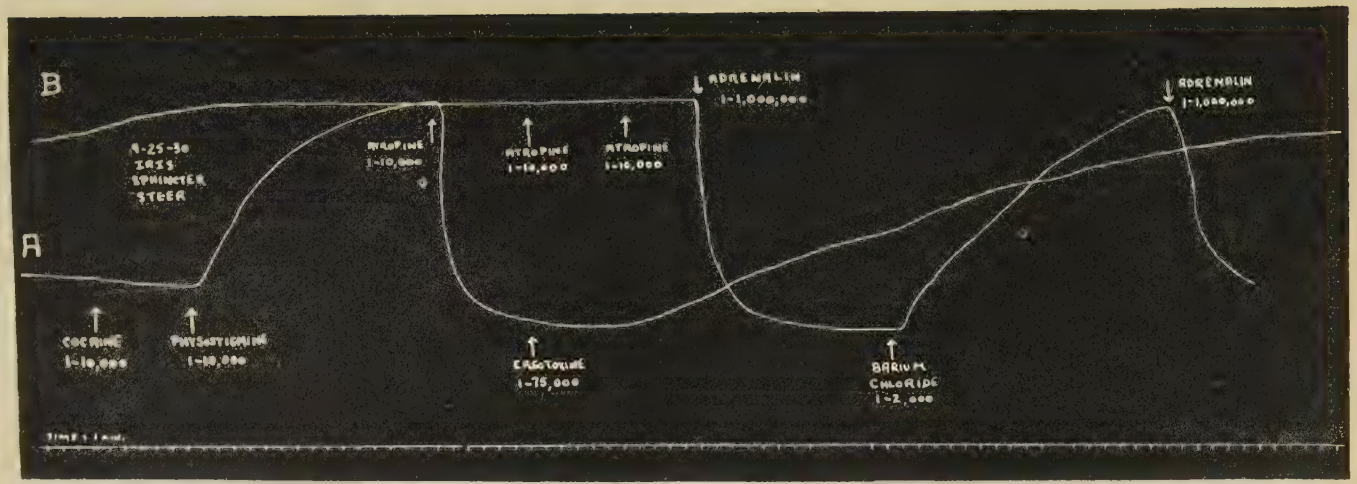

FIG. 1.

Continuous composite tracing of effects of various drugs on an isolated strip of iris sphincter muscle of steer's eye.

At A, drugs added in order, cocaine, 1-10,000; physostigmine, 1-10,000; atropine, 1-10,000; ergotoxine, 1-75,000; and at B (continued around the drum), atropine, 1-10,000 (twice); adrenalin, 1-1,000,000; barium chloride, 1-2,000; and adrenalin, 1-1,000,000.

Drum Speed = 5 mm. per minute.

siderable degree. Ergotamine tartrate produces the same effect as ergotoxine. However, the results do not support the conclusions of Zunz,[3] Hess[4] and Poos[5] that ergotoxine causes this increased tonus by the parasympathetic route. Atropine in considerable concentration fails to "break through" the tonus elicited by ergotoxine and it does not prevent this tonus increase if applied before ergotoxine (Fig. 1).

In the intact eye of 7 cats, after recovery from light ether, ergotoxine miosis, produced by Koppanyi's intraocular method, was partially lessened by atropine intraocularly. The pupil of the eye thus treated did not correspond in size to the normal pupil of 7 mm. but was several millimeters smaller, indicating a direct action of ergotoxine on the iris sphincter muscle. (See Table I.)

TABLE I.

Cat—Male—Wt., 3.5 kg. Normal pupils = 7 mm. Light reflex present.

Time	Right P.	Left P.	Remarks
10:53 a. m.	7 mm.	7 mm.	Ergotoxine (0.12 mgm.) intraocularly into right eye.
10:58	6	8	
11:13	4	8	
11:19	3.5	8	
11:24	3.5	8	Instilled 0.03 mgm. Ergotoxine into right conjunctival sac.
11:40	2.5	8	Light reflex still present, sluggish return in right eye.
12:05 p. m.	1.8	8.5	
12:40	0.8	8.5	
2:00	Slitlike	8.5	
3:07	0.8 mm.	8.5	Atropine (4 mgm.) subcutaneously.
3:30	0.8	11	Light reflex absent.
3:45	0.8	11	Atropine (10 mgm.) subcutaneously.
4:05	0.8	12	
4:10	0.8	12	Atropine (4 mgm.) intraocularly into right eye.
4:20	1.5	12	Light reflex absent.
4:40	1.5	12	

To Mr. Eugene Marti of the Sandoz Company we are indebted for a liberal supply of ergotamine tartrate.

[3] Zunz, C. R., *C. R. des Séances de la Soc. de Biol.*, 1924, xc, 379.

[4] Hess, W. R., *Klin. Monatsbl. fur Augenheilkunde*, 1925, lxxv, 295.

[5] Poos, F., *Klin. Monatsbl. fur Augenheilkunde*, 1927, lxxix, 222.

5092

An Effective Ascaricide-Hexylresorcinol.

PAUL D. LAMSON, CHARLOTTE B. WARD AND HAROLD W. BROWN.

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Ascariasis is a condition which has been recognized since the earliest medical times, and although it may cause little apparent disturbance the parasites are always a potential source of danger from migration into the various body cavities, from mechanical obstruction of ducts, the air passages, or the intestines, and from chemical intoxication. Between the latitudes of 35° North and 30° South there is a belt of ascariasis around the entire world. In the rural population of certain states of this country which lie in this belt, there is an incidence in certain areas as high as 48%,[1] while in other countries, such as China, as many as 90% of the population of certain regions have been found to be infested with these parasites. A great many substances and concoctions have been used for the removal of these worms, but they have all proven to be either inactive or dangerous. The two in most common use at present are Santonin and Oil of Chenopodium (or its active principle, Ascaridol). Both of these substances have been given to many thousands of patients without intoxication. On the other hand many sudden unexplained deaths have followed their use, which makes it impossible to predict what will be the outcome of their administration. Carbon tetrachloride and tetrachlorethylene, which are both very efficient against hookworm, are relatively ineffective against ascaris and may cause dangerous migration of these parasites.[2] We have, therefore, no safe or effective means of removing ascaris. On this account a search for a non-toxic ascaricide was begun several years ago in this laboratory, and a great number of experiments have been carried out on the physiology and pharmacology of this parasite. Several active substances which might be of practical value have been found by us, among which hexylresorcinol seems most nearly to fulfill the necessary requirements of an ideal ascaricide.

Hexylresorcinol is a white, waxy, crystalline substance. That which we have used in these experiments was kindly given us by Dr. Veader Leonard and had a melting point of 59°-61°C. It is only slightly soluble in water or mineral oil but readily soluble in alco-

[1] Cort, W. W., Otto, G. F., and Spindler, L. A., *South. Med. J.*, 1929, xxii, 608.

[2] Lamson, P. D., Minot, A. S., and Robbins, B. H., *J. Am. Med. Assn.*, 1928, xc, 345.

hol, glycerin and vegetable oils. It has been shown by Leonard to have an extraordinarily high bactericidal action and to be the least toxic of a large series of substituted resorcinols which he has studied.[3] It has been given as the pure crystals in gelatin capsules by mouth in doses varying from 0.1 to 1.0 gm. 3 times a day to individuals for periods of as much as 10 weeks without any deleterious effect,[4] and to many hundreds of patients in olive oil under the name of Caprokol (N.N.R.) as a urinary antiseptic.[5] It has been shown by us to be extremely active in great dilution on the pig ascaris *(Ascaris suum) in vitro* in several hundred experiments; to remove 100% of the ascaris in 16 out of 17 dogs as shown by autopsy, and when given in 1 gm. doses in gelatin capsules to 20 patients harboring ascaris to have removed from 90% to 100% of these parasites, as shown by carefully controlled egg counts. One hundred human cases of ascariasis have been carefully studied and controlled by egg counts before and after the administration of hexylresorcinol in varying amounts, and given in different ways. The results of these experiments will be reported later as well as comparative studies of other resorcinols.

Crystalline hexylresorcinol has irritant properties. It causes a burning sensation of the tongue, followed by an anesthetic action. The crystals, aqueous solutions, or mineral oil suspensions, all give this burning sensation in the mouth and are unpalatable. Although this burning sensation on the tongue can be largely overcome by dissolving the hexylresorcinol in vegetable oils, our experiments show that the action of hexylresorcinol on ascaris *in vitro* is greatly decreased when dissolved in such a solvent. For example, 0.1% hexylresorcinol in water will kill *Ascaris suum in vitro* in 2 minutes, while it takes 20 minutes or more to kill these parasites in a 3% olive oil solution.

Although these purely experimental administrations of one gram of hexylresorcinol crystals in hard gelatin capsules to adults and one-half gram to children followed by mineral oil have been extremely efficient in removing ascaris from dogs and man, the therapeutic dose and best mode of administration remain to be worked out.

The general feeling has been that one must have a vermifuge which would remove all the worms in a single dose. This is important when elaborate preparations for treatment must be made, and

[3] Leonard, Veader, *Science*, 1925, lxii, 408.

[4] Leonard, Veader, *J. Urol.*, 1924, xii, 585.

[5] Leonard, Veader, and Wood, Austin, *J. Am. Med. Assn.*, 1925, lxxxv, 1855.

where there is danger of intoxication. If, however, as is the case with hexylresorcinol, no preparation of the patient is necessary except to take the drug in the morning on an empty stomach and to wait an hour or so before eating to let the drug come in better contact with the parasites, there is no reason why the drug should not be repeated if necessary.

It should be pointed out that although hexylresorcinol crystals have been given by Leonard to many individuals and by us to between 50 and 100 patients without symptoms of any kind and have also been taken by us with nothing more than a burning sensation in the stomach, this may cause gastric irritation. In dogs this varies from a general reddening of the gastric mucosa to small submucal hemorrhages, and even necrosis of the epithelium in places. Microscopic sections show, however, that these lesions are entirely superficial. Examination of the gastro-intestinal tract of dogs 3 days or more after the administration of hexylresorcinol crystals shows no evidence whatever of any lesion having been produced. The fact that hexylresorcinol is a strong protein precipitant leads us to believe that it will not penetrate deeply into the tissues. Microscopic examination shows that this is the case and the rapid healing of these lesions, and absence of symptoms in patients having been given this substance in large amounts over long periods of time confirm this belief. If, however, alcohol is taken together with hexylresorcinol, this acts as a solvent and carries the substance deeply into the tissues. Here there is danger of serious damage to the epithelium and alcohol should be strictly avoided when taking hexylresorcinol.

Since an olive oil solution of hexylresorcinol is not a suitable form of administration as an ascaricide, the question naturally arises as to whether one should consider it safe to give the crystals which may cause gastric irritation. Further experimentation may produce a method of administering crystalline hexylresorcinol which will allow it to act on the parasites without causing irritation of the mucosa but it is far safer to consider that such irritation may occur rather than to disregard it. If one considers the action of most drugs it will be seen that our confidence in them is based on the results of their action rather than on our knowledge of how they act. The effect of ether, for example, on the body tissues is invisible yet the functional changes are profound. Consciousness is completely obliterated, an extreme acidosis is produced, as well as hyperglycemia, etc. Its use is justified only because experience has shown that recovery takes place without permanent harm. One also submits to all manner of physical injuries such as sunburn, operations on vital

organs, etc., knowing that regeneration will take place without permanent injury. We feel justified, therefore, in suggesting crystalline hexylresorcinol as an ascaricide in spite of its irritant properties, because we feel that it is exceptionally effective, it kills rather than anesthetizing the parasites, thus reducing the danger of migration, its irritant action, which should theoretically be merely superficial, has been shown by experiment to be so, and finally because hundreds of patients have taken this substance in olive oil solution as a urinary antiseptic in much greater amounts than we suggest for ascariasis without known complications. When given as an ascaricide, it should be taken on an empty stomach to prevent the possibility of its combination with the proteins of the food. Although Leonard has been unable to show any pathological changes after its long continued use the general pharmacology of this substance remains to be worked out.

We wish to express our gratitude to the International Health Division of the Rockefeller Foundation for their generous support of this work, to Mrs. Elfreda Caldwell for her help in the clinical studies, to Dr. E. L. McCafferty and Dr. W. D. Partlow of the Bryce and Searcy Hospitals, Alabama, and finally to Dr. E. L. Bishop, Commissioner of Health for the State of Tennessee, and Dr. F. B. Clark of Gainsboro, Tennessee, for their kindness in making arrangements for the clinical study of this drug.

5093

The Accumulation of Iron in Tuberculous Areas.

VALY MENKIN.*

From the Henry Phipps Institute, University of Pennsylvania, Philadelphia.

In previous communications[1, 2, 3] it was shown that a vital dye, trypan blue, or a metal in the form of its salt, ferric chloride, when injected into the circulating blood stream rapidly accumulates in an area of inflammation, where the substance is fixed and fails to drain to the tributary lymph nodes.

Bowman, Winternitz, and Evans by microscopic studies[4] found

* Fellow in Medicine, National Research Council.

1 Menkin, V., *J. Exp. Med.*, 1929, l, 171.

2 Menkin, V., *J. Exp. Med.*, 1930, li, 879.

3 Menkin, V., and Menkin, M. F., *J. Exp. Med.*, 1930, li, 285.

4 Bowman, F. B., Winternitz, M. C., and Evans, H. M., *Centralbl. Bakteriol.*, 1912, lxv, 403.

that, in experimental tuberculosis, trypan blue injected intravenously stains tubercles in the liver. They pointed out the great affinity of giant and epithelioid cells for this vital dye, which was always found as granules within these cells. Lewis[5] found that if the cornea of a rabbit is inoculated with a living culture of the tubercle bacillus, a progressive lesion results characterized by an intense congestion of the conjunctiva. If the animal is given an intravenous injection of trypan red 24 hours or more after such inoculation, the fluid in the anterior chamber of the inoculated eye always becomes colored.

In view of the observation that iron, intravenously injected, accumulates in inflamed areas, a few experiments were set up to determine whether this metal when injected into the circulating blood stream would accumulate and be detected in tubercles.

Bovine tuberculosis was induced in several rabbits by the intravenous injection of 0.001 mg. of a saline suspension of Ravenel strain. From 4 to 5 weeks later extensive tuberculosis had developed in the lungs of most of the animals. These rabbits were given several daily intravenous injections each consisting of 5 cc. of a 0.25% solution of ferric chloride crystals. Three animals were killed after 3, 4 and 7 injections of ferric chloride. The lungs were carefully removed and dipped in acidified potassium ferrocyanide (3 parts of 1% HCl to one part of 2% $K_4FE(CN)_6$).

After an interval ranging from 45 minutes to 1 hour or sometimes less the tubercles showed a marked Prussian blue reaction on gross examination. The blue color was intense in the caseous or central part of the tubercle. The dye or iron that penetrated from the blood stream into the periphery of an inflamed area (loc. cit. 1, 2) on the contrary failed to enter the central part in which the circulation is relatively inactive. It is noteworthy in this connection that various dyes that cannot penetrate living cells stain dead or dying cells.[6]

Gierke[7] pointed out many years ago that calcified tubercles in the lungs fail to react to the microchemical test for iron. As control to the observations described above, several tuberculous animals that had not been injected with iron were killed by ether anesthesia a few weeks after infection with the bovine microorganism. The lungs were immediately tested for the presence of the metal in the tubercles by the Prussian blue reaction. The results in this group of animals were negative. It was also found that a single injection of

[5] Lewis, P. A., *J. Exp. Med.*, 1916, xxiii, 669.

[6] Steckelmacher, *Beitr. Path. Anat.*, 1914, lvii, 314.

[7] Gierke, *Virchow's Arch.*, 1902, clxvii, 318.

20 cc. of 0.25% solution of ferric chloride produced no demonstrable Prussian blue reaction in the tubercles.

Further experiments are being conducted to substantiate these preliminary results and to determine the effect of the accumulation of iron in tuberculous areas on the course of development of the disease.

5094

Complete Atrophy of Kidney in Pigeons Following Section of the Ureter.

OSCAR RIDDLE.

From the Carnegie Institution, Station for Experimental Evolution.

In a publication dealing with effects of extirpation of the *bursa Fabricii* in young doves Riddle and Tange[1] showed that this operation sometimes involves the section of one or both ureters. Among 31 operated animals which they reared to maturity 2 individuals were found at autopsy to have each only a single kidney. It was concluded that "the section of the right ureter probably caused the complete disappearance of the right kidney in these 2 animals." The earlier study was carried out on ring doves. More recently Riddle, Krizenecky and Polhemus (unpublished) have bursectomized and thymectomized a series of young common pigeons as part of a study of possible functions of bursa and thymus in relation to sex and reproduction. Incidental to that study 3 additional cases of absent or partially atrophied kidneys have been found. It now seems possible to demonstrate that the section of a ureter in young doves and pigeons is often followed by (a) its occlusion at the point of cutting, (b) the accumulation of pressure in this ureter and in the corresponding kidney, and (c) the partial or complete disappearance of the kidney tissue.

The principal facts are presented in Table I. Cases 1-3 were obtained in ring doves; cases 4-6 in common pigeons. Case 3 is particularly interesting since it indicates the rapidity of the changes in the kidney. Only 20 days after the right ureter was cut this duct was found to be bulbous and much distended throughout its course from the kidney to its blind end, at the point of cutting, near the cloaca; the right kidney had evidently undergone considerable atrophy and

[1] Riddle, O., and Tange, M., *Am. J. Physiol.*, 1928, lxxxvi, 266.

TABLE I.
Partial or Complete Atrophy of Kidney After Section or Tearing of Ureter.

Case	Age (days) at		Description of right or left:	
	Operation	Autopsy	Ureter	Kidneys
1	48	410	R. much distended	R. absent; L. enlarged
2	30	294	R. " "	R. " ; L. "
3	83	103	R. " "	R.=0.238g.; L.=0.618g.
4	57(86)	237	R. bulbous, distended	R.=0.347g.; L.=1.525g.
5	58(89)	369	L. " "	L.=0.032g.; R.=1.818g.
6	59	195	L. sausage-like; dist.	L. absent; R.=(1.700g.)

the left kidney had evidently already increased in weight. The expected or normal size of each kidney is 400-500 mgm. in the ring doves, and 700-1000 mgm. in common pigeons.

It is notable that though all of the ureters described in the table were much swollen and distended by fluid this *fluid contained no visible insoluble urates in any instance.* It is practically certain that an earlier accumulation of uric acid had disappeared during the relatively long interval (20-362 days) between operation and autopsy. The following case provides part of the evidence: A common pigeon was bursectomized at 58 days; it died 3 days later, apparently of uremia. Examination showed that both ureters had been cut and that the cut surface had quickly healed and closed the cloacal ends of both ureters; there was no escape of fluid to the body cavity. Both ureters were here markedly distended with fluid and semifluid, plainly containing the usual insoluble excretions of the bird. Both kidneys, at this stage, were plainly enlarged and irritated.

The 3 cases (4-6) of atrophied kidney in common pigeons were obtained from 20 of these animals which were operated (bursectomized) and reared to maturity. On cases 4 and 5 a second or exploratory operation was performed when these birds were 86 or 89 days old. In neither case was any bursa tissue found or removed at the second operation, but is is possible that a ureter was cut or torn at this time, instead of at the first operation. From 51 young birds operated and reared to maturity (case 3, not mature) 5 cases of kidney atrophy were obtained. In 3 of these cases the kidney completely disappeared, leaving not even the trace of a blood vessel in the clean bony case which had been the site of the organ.

Aside from some obvious interest to the pathologist these cases have a bearing on the problem of agenesis of organs. The occasional unexpected absence of an organ, in either embryo or adult, usually raises the question as to whether it is a case of non-formation (agenesis)—a truly developmental or genetic matter—or of disappearance after formation. In the case of the kidney the absence of one of the

normal pair has been repeatedly observed in man and in various animals. We have observed 3 such cases in unoperated pigeons—2 from the genus *Turtur,* and one from *Streptopelia.* In view of the results described in this paper it is certainly possible that some or many of the cases of absent kidneys observed in various animal species are not true cases of agenesis, but of atrophy following the malformation, occlusion, or malfunction of the ureter attached to the missing organ. It seems, in view of observations to be mentioned immediately, that precisely such glandular structures as the kidney would undergo such atrophy more frequently than would other organs. The same series of operations which resulted in the cutting of a few ureters (in surviving birds), resulted also in the section and occlusion of twice as many sperm ducts in the 20 males used; *but in no single instance was there a resulting atrophy of the testis.* In most cases the epididymis was enlarged, it was cystic in 3 cases, but the testes showed no tendency to decrease in size. In the females some oviducts were cut, and though this prevented successful egg-laying, their ovaries were nearly or quite normal. We have earlier reported[2] 16 cases of birds with complete absence of gonads, and the cause (or history) of these absent organs is of theoretical importance. The present results show that one means of effecting kidney atrophy in these animals is wholly without such action on the gonads.

Summary. Atrophy, partial and complete, of the kidney of dove or pigeon can be obtained by section of one ureter. Pressure develops in the occluded duct and kidney, and insoluble urates later disappear from the contents of the duct. The uninjured kidney readily undergoes functional hypertrophy.

These observations have a bearing on the problem of the agenesis of organs. They indicate that in some cases the absence of a glandular organ which, like the kidney, is functional in the embryo may have been formed (not agenesis) and—through malformation or malfunction of its duct—later undergone complete atrophy. In contrast, section of the genital ducts of these birds was not followed by atrophy of testis or ovary; this evidence therefore indicates that the absence of gonads in birds has a different origin and significance.

[2] Riddle, O., *Brit. J. Exp. Biol.,* 1925, ii, 211.

5095

Effect of Drying in Air on the Goiter-Producing Substance in Cabbage.

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From the Laboratory Division, Montefiore Hospital, New York.

It has been shown that fresh cabbage, steamed cabbage and cabbage from which 60% of its weight has been removed as press juice produce thyroid hyperplasia in rabbits when fed as their principal food.[1]

It has also been shown that steamed hashed cabbage that has been kept exposed to air in a moist state for 4 to 5 days fully retains its goiter-producing quality. The goitrogenic substance was therefore heat stable and at least not readily lost when exposed to air in the moist state. As a further step in determining the nature of the goitrogenic substance in cabbage we have studied the effect of drying. No facilities were available for drying cabbage *in vacuo* in the amounts needed. Experiments were made with tested goitrogenic cabbage dried in 2 ways as follows:

1. Imported Holland winter cabbage was steamed for 30 minutes. The juice was pressed out under 450 lb. pressure and the pressed cake was then fed into an atmospheric double drum drier. Steam was introduced into the drums under 75 lb. pressure. The cabbage was in contact with the drums about 30 seconds and was automatically scraped off as nearly dry flakes. This material was at once transferred to covered containers in contact with solid CO_2 and kept over night, when it was stored in flasks and evacuated.

2. Northern New York winter cabbage (batch 21) was steamed for 35 minutes and 50% of its weight was removed as press juice. The leaves were then separated and put in trays in a current of air at room temperature. Drying was completed in about 36 hours and the product represented from 7 to 9% of the original whole steamed cabbage.

Both of these dry preparations were moistened with water before feeding and given to rabbits in proportion to body weight. The rabbits ate it readily, gained weight and maintained a sleek healthy

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[1] Marine, D., Baumann, E. J., and Cipra, A., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 822.

appearance. Control rabbits were fed with steamed pressed cabbage cake from the same lot of cabbage without drying.

The principal data are given in Table I.

TABLE I.

Sex	Weight at Beginning	Preparation Number	Amount Fed per day gm.	No. days Fed	Weight at End	Condition of Thyroid
M	1200	1	41*	26	1365	Normal
F	1128	1	38*	26	1227	Normal
M	2260	2	53*	21	2206	Normal
F	2300	2	53*	21	2351	Normal
F	1845	Control	214* Pressed cake	18	1703	Moderate hyperplasia
F	2290	Control	266* Pressed cake	18	2175	Moderate hyperplasia

* Supplementary diet of 17 gm. oats and 20 gm. alfalfa hay twice weekly.

These experiments indicate that cabbage loses its goitrogenic quality when dried by the methods indicated.

5096

Effect of Acid and Alkaline Hydrolysis on the Goitrogenic Substance Contained in Cabbage.

BRUCE WEBSTER* AND ANNA CIPRA.

From the Laboratory Division, Montefiore Hospital, New York.

Further studies have been carried out in an effort to determine the nature of the goiter-producing substance contained in cabbage and other vegetables.[1, 2]

It was first considered advisable to attempt to find out whether or not this goiter-producing factor was soluble in water. Cabbage was steamed for 30 minutes, hashed and pressed until 50% of the total weight had been removed. The residue thus obtained had previously been shown[2] to contain a considerable proportion of the goiter-producing substance. Samples of this residue were washed with one and two volumes of water respectively, at room temperature and the water removed by pressing. As shown in the accom-

* Fellow of the National Research Council.

[1] Webster, Bruce, and Chesney, A. M., *Am. J. Path.*, 1930, vi, 275.

[2] Marine, D., Baumann, E. J., and Cipra, Anna, PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 822.

panying table, no marked change in the goiter-producing power occurred in either instance. Similarly, boiling the above residue for 15 minutes in 2 volumes of water, and pressing to its original weight did not materially alter its goitrogenic power.

Studies were then made of the effect on the goitrogenic power of cabbage of extracting with water at various hydrogen ion concentrations. The fraction which remained, after removal of 50% of the total weight as juice, from steamed hashed cabbage, was allowed to stand for 15 minutes at room temperature in 2 volumes of water to which sufficient hydrochloric acid had been added to bring the pH of the mixture to 3.0. The water was then pressed

TABLE I.

Rabbit No. and Sex	Cabbage Lot No.	Method of Treatment of Cabbage Cake	Duration of Feeding in Days	Condition of Thyroid Gland at Termination
847 F	21	Extracted for 15 minutes	23	Moderate hyperplasia
848 F	21	at room temperature with 2 vol. water. Pressed to original weight.	23	" "
865 M	21	Boiled for 15 minutes	21	" "
866 F	21	with 2 vol. water. Pressed	21	" "
863 M	22	to original weight.	21	" "
902 M	22	"	21	" "
759 F	9	Extracted with 2 vol.	18	Marked "
791 F	9	water at pH 3.0 at room	23	" "
826 M	9	temp. for 15 minutes. Pressed to original wgt.	23	Moderate "
849 F	21	Boiled with 2 vol. water	23	Marked "
850 F	21	at pH 3.0 for 15 minutes.	23	" "
869 M	21	Pressed to original wgt.	21	" "
870 F	21	"	21	" "
847 F	23	"	19	" "
903 M	23	"	19	Moderate "
871 M	21	Boiled with 2 vol. water	21	Marked "
872 F	21	at pH 3.0 for 30 minutes. Pressed to original wgt.	21	" "
873 M	21	Boiled with 2 vol. water	21	Moderate "
874 F	21	at pH 3.0 for 60 minutes.	21	
773 M	21	Pressed to original wgt.	21	" "
885 M	21	"	21	" "
851 F	21	Extracted with 2 vol.	23	Slight "
852 F	21	water at pH 9.0 at room temp. for 15 minutes. Pressed to original wgt.	23	" "
867 M	21	Boiled with 2 vol. water	21	Moderate "
868 F	21	pH 9.0 for 15 minutes. Pressed to original wgt.	21	Slight "
853 F	21	Control. Untreated cab-	21	Moderate "
854 F	21	bage cake.	21	" "
861 M	22	"	21	" "
901 M	22	"	21	Slight "

All animals in this experiment were given a supplementary diet of 17 gm. oats and 20 gm. alfalfa hay twice weekly.

out and the residue fed to rabbits. Such a procedure invariably produced a definite increase in the goiter-producing power of the cabbage. Boiling the cabbage in 2 volumes of water at pH 3.0 for 15 minutes further increased its goitrogenic power. Boiling at pH 3.0 for 30 minutes gave no detectable improvement over boiling for 15 minutes. Boiling for one hour, on the other hand, tended to decrease, but not to destroy entirely, its ability to produce thyroid hyperplasia. The results of the above experiments are summarized in the accompanying table. The feeding of equivalent amounts of hydrochloric acid to control animals did not produce goiter.

Attempts were made to study the effect of extracting the cabbage at pH 9.0. Since it has been shown[3] that the feeding of excessive amounts of alkali will, in itself, produce thyroid hyperplasia in animals, the results of our experiment are difficult to interpret. However, it appeared that extraction of the cabbage at pH 9.0 tended to lessen its goitrogenic power.

The results of these experiments would suggest that the goiter-producing substance contained in cabbage and other vegetables is not readily soluble in water, either at room temperature or at 100°C. Further, mild acid hydrolysis does not extract this substance. Prolonged acid hydrolysis at high temperatures may extract or destroy it to a slight degree. Alkaline hydrolysis appears to extract or destroy the goiter-producing factor.

The apparent increase in goitrogenic activity after contact with acid, may be due to a preservation of the goitrogenic substance, or on the other hand, it may be due to the destruction or extraction of anti-goitrogenic agents.[4] Further experiments along these lines are in progress.

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[3] Marine, D., unpublished data.

[4] Marine, D., Baumann, E. J., and Webster, B., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 1029.

5097

Occurrence of Antigoitrogenic Substances in Plant Juices.

DAVID MARINE, EMIL J. BAUMANN AND BRUCE WEBSTER.*

From the Laboratory Division, Montefiore Hospital, New York.

The discovery by Chesney, Clawson and Webster[1, 2, 3] that cabbage when fed to rabbits as their principal food produces marked thyroid hyperplasia has provided a rapid and more practical method for testing various antigoitrogenic agents. Last year we showed that cabbage grown in the spring and early summer is much less goitrogenic than cabbage maturing in the late autumn.[4] Also it was pointed out that whole press juice and juice concentrates made from potent goiter-producing cabbage had little or no goitrogenic activity.

Further work showed that the goitrogenic activity of cabbage was in general inversely proportional to its ability to absorb iodine. This suggested that there were 2 substances in cabbage in variable amounts, one of which was goitrogenic and the other antigoitrogenic. This view is further supported by the fact that washing hashed, steamed cabbage with water increases its goitrogenic activity. Since the antigoitrogenic activity corresponds roughly to the amount of reducing substance (determined iodometrically), we have sought an available plant which contained the reducing substance in greater amounts than is ordinarily found in common vegetables. We have obtained very potent concentrates from the juice of skunk cabbage *(Symplocarpus foetidus)* and have found that it produces thyroid involution in rabbits in from 8 to 10 days when fed by mouth daily in amounts equivalent to 100 units (a unit being the amount of juice required to absorb one cc. of N/100 iodine) in addition to our standard stock diet of oats and alfalfa hay. Sterile fractions introduced intraperitoneally appear to be more effective. Fresh cut alfalfa and lawn grass also produce involution of thyroid hyperplasia when fed to rabbits in amounts of 300 to 400 gm. daily for 3 weeks. The early spring growth of all of these plants contains large amounts of the iodine-absorbing material. Plant juices which are extremely low in the reducing substance have little or no

* Fellow of the National Research Council.

1 Chesney, A. M., Clawson, T. A., and Webster, B., *Johns Hopkins Hosp. Bull.*, 1928, xliii, 261.

2 Webster, B., Clawson, T. A., and Chesney, A. M., *Johns Hopkins Hosp. Bull.*, 1928, xliii, 278.

3 Webster, B., and Chesney, A. M., *Johns Hopkins Hosp. Bull.*, 1928, xliii, 291.

4 Marine, D., Baumann, E. J., and Cipra, A., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 822.

antigoitrogenic action. This antigoitrogenic substance is present in a great variety of plants but in highly variable amounts as regards age, species and climate.

Whether this reducing substance is identical with that which Szent-Györgyi[5] isolated from cabbage, orange juice and suprarenal cortex and found to be a hexuronic acid has not been established. All the evidence at present indicates that this reducing substance is at least one of the substances causing thyroid involution. The facts that a similar reducing substance occurs in the suprarenal cortex, the corpus luteum and the gonads and that we[6] have demonstrated that extracts of the suprarenal cortex have an inhibiting or regulatory effect on thyroid activity also support the view that the reducing substances in plants which involute thyroid hyperplasia belong to the same group of compounds which Szent-Györgyi isolated from the suprarenal. There is also evidence that this reducing substance hastens thymus involution and increases the weight of the suprarenals in rabbits. The involuting effect of these reducing substances is not due to iodine.

The iodine content of sheep, beef and hog thyroids as shown by Seidell and Fenger[7] is lowest in the early spring months. On the other hand the incidence of goiter in animals is highest during this season of the year when the amount of reducing substances in stored foods is at its lowest level. As the animals obtain fresh green food, the iodine content of the thyroid rises. Up to the present this rise in the iodine store has been assumed to be dependent upon an increased intake of iodine, but from the observations we have made it appears probable that the iodine store rises because the thyroid hormone is less needed. The relation of the suprarenal glands and gonads to thyroid enlargement also becomes more understandable in the light of these experiments. The suprarenals and gonads are normally a storehouse and possibly a place of manufacture of the reducing substance and so long as the supply of reducing substance is available in ample quantities it exerts a sparing action on the thyroid. We have repeatedly pointed out, in discussing the relation of iodine to thyroid enlargement,[8] that one must recognize those enlargements dependent upon an absolute deficiency of iodine (endemic goiter) and those dependent upon a relative deficiency (sporadic goiter), that is, due to conditions which increase the needs of the body for iodine as occurs in the thyroid enlargement of puberty,

[5] Szent-Györgyi, A., *Biochem. J.*, 1928, xxii, 1386.

[6] Marine, D., Baumann, E. J., and Cipra, A., *Am. J. Physiol.*, 1925, lxxii, 248.

[7] Seidell, A., and Fenger, F., *J. Biol. Chem.*, 1913, xiii, 517.

[8] Marine, D., *Medicine*, 1927, vi, 127.

pregnancy, Graves' disease and other associations. One of the conditions producing the relative iodine insufficiency now appears to be a deficiency in the reducing substance.

It is our present belief that plant juices prevent or cure thyroid hyperplasia by a thyroid-sparing acting, that is, by providing another mechanism for promoting tissue oxidations. On the other hand, iodine administration prevents thyroid hyperplasia by making it easier for the thyroid to produce more thyroxin.

5098

Action of X-rays on Glutathione Content and Oxygen Consumption of Normal and Regenerating Planarians.*

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Studies upon regeneration[1] have been carried out to test the hypothesis that persistent embryonic cells are responsible for the formation of new tissues during regeneration, and that the power or capacity for regeneration is to be correlated with the number of such cells present. It has been shown that in different species of planaria, cells of an embryonic type, called "formative cells," undergo rapid proliferation after cutting the planarian, migrate to injured areas, and differentiate into the new tissues characteristic of the regenerating part. Species of planarians that exhibit the greatest capacities for regeneration possess the greatest numbers of these formative cells. Hickman[2] destroyed the capacity for regeneration by the use of X-rays and radium, and histological studies of such irradiated worms showed a selective destruction of these formative cells.

Other investigators showed that the rate of metabolism and of oxygen consumption in particular, increase immediately after cutting and remain at a high level throughout regeneration. The important rôle of glutathione in the establishment of the metabolic level and in the utilization of oxygen indicated that important cor-

* Acknowledgements are due the Committee on Effects of Radiation, National Research Council, for support received through W. C. Curtis.

1 Curtis, W. C., *Proc. Boston Soc. Nat. Hist.*, 1902, xxx, 515.

2 Curtis, W. C., and Hickman, J. F., *Anat. Rec.*, 1926, xxxiv, 145.

relations might be found between the processes of regenerative development and the quantitative distribution of this compound. Numerous workers have found glutathione in greater concentrations in the tissues of embryos and in adult tissues capable of continued cell division than in adult tissues in general. Recently Hammett[3] has shown glutathione to be a stimulus to cell division.

Using a modification of methods of Joyet-Lavergne[4] and Hammett[3] the glutathione content was determined in two species of Turbellarians, *Planaria agilis,* and *Procotyla fluviatilis (Dendrocoelum lacteum).* The former species possesses a high capacity for regeneration and shows a high concentration of glutathione; the latter, which exhibits a low capacity for regeneration, shows a lower content. In normal worms the formative cells are found in the parenchyma, principally in the dorsal region. This area corresponds to that showing the highest content of glutathione. In regenerating individuals greater numbers of differentiating formative cells and higher glutathione content are found in the regenerating areas that form the head, tail, and pharynx.

Quantitative examinations of the glutathione content of normal, regenerating, and irradiated specimens of *Planaria agilis* were made by colorimetric estimations and by the Tunnicliffe[5] method. The glutathione content was found to decrease after irradiation with heavy exposures of X-rays (approximately 2,000 R units). Some typical results are found in Table I.

TABLE I.
Glutathione Content of Planarians Before and After X-ray Treatment (in mg. per 100 gm. of tissue).

Animals	Normals	2 hrs. after Irradiation
Pure line R-1-3	73	65
" " R-2-4	86	53
" " R-1-4	79	66
Mixed culture	94	81
Mixed culture	94	81
Average	83	65.75

Determination by Tunnicliffe method.

The oxygen consumption of individual specimens of *Planaria agilis* of the above types has been determined by the Winkler method. A micro-adaptation was devised in which determinations were made on small samples of fluid in an atmosphere of pure hydrogen. The

[3] Hammett, F. S., *Protoplasma,* 1919, vii, 297.

[4] Joyet-Lavergne, Ph., *C. R. Soc. de Biol.,* 1928, xcviii, 658.

[5] Tunnicliffe, H. E., *Biochem. J.,* 1925, xix, 194.

results upon regenerating worms confirm those of other workers. Oxygen consumption remains above normal during regeneration. Oxygen consumption of both normal and regenerating worms is markedly decreased by heavy exposures with X-rays. The profound effect of this treatment on the oxygen consumption may be seen from Table II.

TABLE II.

Oxygen Consumption (cc. per hr.) of Individual Planarians Before and After X-ray Treatment.

Animals	Normals before irradiation	24 hrs. after irradiation
A	0.01472	0.00114
B	0.00924	0.00027
C	0.00876	0.00110
D	0.00559	0.00037
E	0.01186	0.00570

Determinations by a micro-modification of the Winkler method.

In view of the stimulating action of glutathione upon cell division and the high concentration of this compound in embryonic cells, the destruction of glutathione by X-rays or at least its inactivation offers an explanation of the selective action of X-rays upon cells undergoing mitotic division and upon embryonic cells of planarians and other forms.

5099

Action of Adrenalin on the Metabolism of Peripheral Tissues.

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From the Department of Physiology, University of Buffalo Medical School.

It has been found impossible to affect the carbon dioxide or total acid production of excised frog muscles by placing them in adrenalin solutions.[1] The absence of a calorigenic effect[2] under such unphysiological conditions was not surprising; and more recent work has indicated that the absence of the viscera[3] and particularly of the liver[4] may have been as responsible for the negative result as the

1 Griffith, *Am. J. Physiol.*, 1923, lxv, 15.

2 Boothby and Sandiford, *Am. J. Physiol.*, 1923, lxvi, 93.

3 Soskin, *Am. J. Physiol.*, 1927, lxxxiii, 162.

4 Casky, *Am. J. Physiol.*, 1927, lxxx, 381.

lack of proper circulation or other abnormal conditions to which the muscles themselves were immediately exposed.

It is not known in what manner the viscera cooperate in the calorigenic action of adrenalin; whether by serving as the locus of its action, or by keeping the peripheral tissues in the proper physiological condition to respond to the adrenalin itself. The following experiments were undertaken to determine some of the effects that might be produced in one of the hind legs of an anesthetized cat upon the addition of a known amount of adrenalin directly to its arterial blood supply.

All experiments were done on cats under chloralose anesthesia. The animal was always prepared so that simultaneous samples could be taken of the arterial blood going to and venous blood coming from the left hind leg. At the time of collection of the venous sample the rate of flow was also determined in a manner somewhat similar to that described by Himwich and Castle.[5] The usual precautions were taken to prevent alteration in the gas content of the blood; and this was determined by the manometric method of Van Slyke and Neill, using 0.2 cc. samples in duplicate.

In large cats that could stand the additional loss of blood, 2 normal sets of samples, 10 or 15 minutes apart, were taken before the adrenalin injection; these served to establish the average spontaneous variation to be expected under the conditions of these experiments. In the remaining experiments the adrenalin was injected immediately after taking a single normal pair of samples.

The adrenalin was made up in 0.9% NaCl or mammalian Ringer from 1:1,000 Parke-Davis adrenalin chloride solution, neutralized and warmed before injection; and this was made directly into the iliac artery so that the adrenalin acted first, and we believe, in most cases, only on the tissues of the leg under observation.

Results: As controls we have 18 pairs of normal samples taken 10-15 minutes apart before injecting adrenalin; the average of these gives for the rate of blood flow and oxygen consumption of the first and second samples, respectively, 30.9 and 1.77; and 30.1 and 1.74 (all are cc. per minute). In other words in the 10 to 15 minutes elapsing between the first and second blood samples the rate of blood flow decreased only 0.8 cc. per minute and the oxygen utilization only 0.03 cc. per minute.

The effect of injecting adrenalin at the rate of 0.0004 mg. per cc. per minute for 5 minutes; we give the average of 10 experiments; in these the rates of blood flow and of oxygen consumption just

[5] Himwich and Castle, *Am. J. Physiol.*, 1927, lxxxiii, 92.

before the injection were, in cc. per min., 29.0 and 2.05; just after the injection they were, 26.0 and 1.93, *i. e.*, the adrenalin decreased the blood flow 3.0 cc. and the oxygen utilization 0.12 cc. per minute. We have another set of 9 experiments in which the adrenalin was given at just twice the rate used above, *i. e.*, 0.0008 mg. per cc. per minute for 5 minutes; the average rate of flow and of oxygen consumption just before these injections was 33.8 and 2.44, respectively; just after, the figures are, 27.6 and 2.14; *i. e.*, this dose of adrenalin slowed the rate of blood flow by 6.2 cc. per minute and the oxygen utilization 0.30 cc. per minute.

Although these decreases of oxygen utilization are not large they are 4 and 10 times greater than the change observed to occur spontaneously during an interval 2 or 3 times as long between normal samples; but even more significant, perhaps, is their constancy. Of the 18 pairs of normal samples, the oxygen consumption of the second was less than the first in 11 and greater in 7; of the 19 adrenalin experiments the oxygen consumption following the injection was less than the normal in 14, unchanged in 1, and greater in only 4.

These figures are derived from uncorrected oxygen contents; the determination of the oxygen capacity of 33 pairs of arterial-venous pairs gave an average venous capacity 0.46 vol. %, less than the arterial; this is not only opposite in sign to what has previously been reported (see 5) but also cannot be due to changes in cell volume, which, as an average of 27 determinations, was 55.07 for arterial, and 54.98 for the venous samples.

The effect of adrenalin on the carbon dioxide production was too variable and uncertain to justify speaking of it until further work is done.

5100

Dynamics of Insulin Secretion by the Pancreas and Epinephrine Secretion by the Suprarenal Gland.*

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Many authors have tried to prove that the pancreatico-duodenal vein is the carrier of the pancreatic hormone but nobody was able

* This paper has been partly supported by the Committee for Chemistrysation of SSSR, Moscow.

to accomplish this for two reasons: (1) Blood was taken in artificial conditions of acute experiment;[1] (2) for detection of insulin no direct biological, but only unspecific and indecisive indirect tests[2] were used.

We obtained the blood from the pancreatico-duodenal vein of healthy dogs under quite normal conditions by means of angiostomical tubes.[3] For detection of insulin we used the most sensitive biological test of Brugsch and Horsters[4]—hypoglycemic reaction of blood accompanied sometimes by paresis or paralysis of the legs of fasting white mice after intraperitoneal injection of the analyzed blood.

In our experiments the blood drawn from the pancreatico-duodenal vein of fasting angiostomised dogs before and after intravenous injection of 25-50 gr. of glucose was introduced in amounts from 0.2 to 1.0 cc. into the peritoneal cavity of white mice. Two to 3 hours after injection the mice were killed by decapitation and their blood sugar evaluated by the method of Hagedorn-Jensen. The results show in a definite way that in a fasting dog insulin cannot be detected in the pancreatico-duodenal blood even with very sensitive biological tests. After intravenous injections of glucose considerable amounts of insulin are present in the blood of the pancreatico-duodenal vein. There was in literature an uncertainty whether insulin is secreted in an active or in an inactive state; our experiments prove that it is secreted into the bloodstream in an active state.

Contrary to the insulin secretion of the pancreas the epinephrine secretion by the suprarenal glands seems to proceed continually in a fasting dog. We have successfully applied the Brugsch insulin test in our experiments for the detection of epinephrine in blood, hyperglycemic reaction resulting instead of the hypoglycemic one.

The hyperglycemic effect of the blood of the lumbalo-suprarenal vein of a fasting dog injected into the peritoneal cavity of fasting white mice proved to be very characteristic. After injection into the peritoneal cavity of white mice of the same amounts (1 cc.) of the blood taken from the femoral vein of the same dog there could be found no rise of the blood-sugar level.

The amounts of insulin contained in the pancreatic vein after injection of sugar amounts to approximately 0.01 unit per 100 cc., whereas the amount of epinephrine found in the lumbalo-suprarenal vein amounts to 0.00001%.

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[1] La Barre, *C. R. Soc. Biol.*, 1929, ci, 144; 1927, xcvii, 1801.

[2] Diedrich, *Arch. exp. Path. und Pharm.*, 1922, cxxv, 336.

[3] London, E. S., *Harvey Lectures*, 1927-28, 288.

[4] Brugsch und Horsters, *Arch. exp. Path. und Pharm.*, 1930, cxlviii, 295.

5101

Effect and Mode of Action of Tartrates in the Human Body.

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Though tartrates have long been used in medicine, and grapes and grape products, the chief sources of tartrates are common foods, there is still much difference of opinion as to the effect and mode of action of tartrates in the human body. Among the unsettled questions are: 1. Are tartrates absorbed from the intestine? 2. If absorbed, are they oxidized in the tissues? 3. Are they alkalinizing? 4. If alkalinizing, how is this effect produced? Solis-Cohen and Githens,[1] Blatherwick,[2] Post,[3] and others believe tartrates are alkalinizing, the basis of their belief evidently being the idea of absorption and oxidation. Pickens and Hetler,[4] Wood,[5] Simpson,[6] Rose,[7] and others are on the contrary side of the alkalinizing question. Sollmann,[8] McGuigan[9] and others take a middle ground.

We can not tell whether tartrates are absorbed from the human intestine or not; but if they were absorbed in the case of any of our experimental subjects they were evidently oxidized, for we found no tartrates in the urine after their ingestion in quantities as great as 2 U. S. P. doses in a day's intake.

Rochelle salts, grapes, raisins and grape juice all proved to be definitely alkalinizing. Any of these substances, when added to an acid-forming diet, markedly decreased urinary acidity.

This alkalinizing effect of tartrates would be strong evidence of their absorption and oxidation, were it not for another process that explains this effect. In mixtures of feces and Rochelle salts, incubated at 37.5°C., the tartrate ions were decomposed within 6 to 24 hours. This proved true without exception in 15 cases. Bile and

1 Solis-Cohen, S., and Githens, T. S., "Pharmacotherapeutics, Materia Medica and Drug Action," 1928, 1175 and 1176.

2 Blatherwick, N. R., *Arch. Int. Med.*, 1914, xiv, 409.

3 Post, W. E., *J. Am. Med. Assn.*, 1914, lxii, 592.

4 Pickens, L. M., and Hetler, R. A., *J. Home Econ.*, 1930, xxii, 44.

5 Wood, H. C., LaWall, C. H., and others, *The Dispensatory of the United States of America*, 21st ed., 1926, 878.

6 Simpson, G. E., *J. Pharm. and Exp. Ther.*, 1925, xxv, 459.

7 Rose, W. C., *J. Pharm. and Exp. Ther.*, 1924, xxiv, 123 and 147.

8 Sollmann, T., "A Manual of Pharmacology," 1928, 47.

9 McGuigan, H. A., "A Textbook of Pharmacology and Therapeutics," 1928, 282.

pancreatin seem unable to decompose tartrate ions. Berkefeld filtrates of feces have this ability to a very limited extent, if at all. Carefully controlled tests with *Escherichia coli*, communior and acidi-lactici, however, have shown that all of these organisms can decompose the tartrate ions in Rochelle salts and produce alkali from this compound by this means.

The alkalinizing effect of tartrates, caused by bacterial decomposition of tartrate ions in the lumen of the intestine and the consequent freeing of easily-absorbable sodium and potassium ions, is probably their chief mode of action in cases where catharsis is not produced.

Our work is still in progress, and more detailed reports are in process of preparation.

5102

A New Synthesis of Aspartic Acid.*

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From the University of California at Los Angeles.

In previous syntheses of aspartic acid the following reactions have been used: The decomposition of acid ammonium malate by heat,[1] the racemization of active aspartic acid[2] and active asparagine,[3] the reaction of maleic or fumaric acid with ammonia,[4] the reduction of oxalacetic ester oxime,[5] the reaction of silver fumarate or potassium acid fumarate with hydroxylamine hydrochloride,[6] the reduction of nitrosuccinic ester,[7] the catalytic reduction and ammonation of oxalacetic acid,[8] and the hydrolysis of the addition compound formed from sodium malonic ester and chloroacetic ester.[9]

*Financial assistance in this work has been received from the research funds of the University of California.

[1] Dessaignes, *Compt. rend.*, 1850, xxx, 324; *Ibid.*, 1850, xxxi, 432. Wolff, *Ann.*, 1850, lxxv, 293.

[2] Michael and Wing, *Ber.*, 1884, xvii, 2984; *Am. Chem. J.*, 1885, vii, 278.

[3] Piutti, *Ber.*, 1886, xix, 1691.

[4] Engel, *Compt. rend.*, 1887, civ, 1805; *Ibid.*, 1888, cvi, 1734; Stadnikoff, *Ber.*, 1910, xliv, 44.

[5] Piutti, *Gaz. Chim. Ital.*, 1887, xvii, 519.

[6] Tanatar, *Ber.*, 1896, xxix, 1477.

[7] Schmidt and Widman, *Ber.*, 1909, xlii, 497.

[8] Knoop and Oesterlin, *Z. physiol. Chem.*, 1925, cxlviii, 294.

[9] Keimatsu and Kato, *J. Pharm. Soc. Japan*, 1929, xlix, 111 and 123.

The present synthesis is believed to be more satisfactory than those previously reported for aspartic acid.

Phthalimido malonic ester, prepared from potassium phthalimide and bromodiethylmalonate, was converted to sodium phthalimido malonic ester by allowing it to react with molten metallic sodium suspended in toluene. A stable addition product was then formed as a dark colored oil by the reaction of chloroacetic ester with sodium phthalimido malonic ester. This product was readily hydrolyzed by hydrochloric acid with the formation of d,l aspartic acid, phthalic acid, carbon dioxide, and ethyl alcohol. Side reaction products are not probable.

The acid hydrolysate was evaporated to precipitate the phthalic acid which was removed by filtration. Hydrochloric acid was removed by the addition of the calculated quantity of silver oxide to the boiling diluted filtrate. After removing the precipitate of silver chloride and evaporating the filtrate to a small volume d,l aspartic acid crystals separated after standing for 3 days. Upon recrystallization from hot, 50% ethyl alcohol these crystals darkened and decomposed over the range, 311-325°C. Within the limits of experimental error they were shown to contain the theoretical quantity of amino nitrogen.

Additional experiments on the reaction of ethylene bromide, ethylene chloride, methylene bromide, beta chloropropionitrile, beta chloropropionic ester, and trimethylene bromide were carried out. In all cases the liberation of halide ions was nearly complete, but only with trimethylene bromide was the expected addition product formed. This reaction has been used by Sorenson[10] for the synthesis of proline.

5103

Use of Phenol Red in the Addis Test of Renal Function.

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Under certain special conditions the rate of urea excretion in the urine is directly proportional to the blood urea concentration so that the ratio $\frac{\text{Urine rate}}{\text{Plasma concentration}}$ becomes a constant.[1] Under these

[10] Sorenson, *Z. physiol. Chem.*, 1908, lvi, 236.

[1] Addis, T., and Drury, D. R., *J. Biol. Chem.*, 1923, lv, 105.

conditions this ratio varies only with the amount of functioning renal tissue[2, 3] and can be used as an accurate method for measuring renal function in man.[4] It has recently been shown[5] in the

TABLE I.

Time of Urine Collection	Urine volume cc./hr.	Excretion		Blood sample	Plasma Concentration		Urine rate / Plasma concentration Ratio:	
		Phenol red mg./hr.	Urea mg./hr.		Phenol red mg./100cc.	Urea mg./100cc.	Phenol red	Urea
11:00-11:20	620	858	1737	11:10	5.80	36.2	149	48
11:20-11:40	300	599	1460	11:30	3.90	28.5	153	51
11:40-12:00	750	360	1366	11:50	3.25	26.2	160	52

[2] Taylor, F. B., Drury, D. R., and Addis, T., *Am. J. Physiol.*, 1923, lxv, 55.
[3] Addis, T., Myers, B. A., and Oliver, J., *Arch. Int. Med.*, 1924, xxxiv, 243.
[4] Addis, T., *Arch. Int. Med.*, 1922, xxx, 378.
[5] MacKay, E. M., and Oliver, J., *J. Exp. Med.*, 1930, li, 161.

5095

Effect of Drying in Air on the Goiter-Producing Substance in Cabbage.

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From the Laboratory Division, Montefiore Hospital, New York.

It has been shown that fresh cabbage, steamed cabbage and cabbage from which 60% of its weight has been removed as press juice produce thyroid hyperplasia in rabbits when fed as their principal food.[1]

It has also been shown that steamed hashed cabbage that has been kept exposed to air in a moist state for 4 to 5 days fully retains its goiter-producing quality. The goitrogenic substance was therefore heat stable and at least not readily lost when exposed to air in the moist state. As a further step in determining the nature of the goitrogenic substance in cabbage we have studied the effect of drying. No facilities were available for drying cabbage *in vacuo* in the amounts needed. Experiments were made with tested goitrogenic cabbage dried in 2 ways as follows:

1. Imported Holland winter cabbage was steamed for 30 minutes. The juice was pressed out under 450 lb. pressure and the pressed cake was then fed into an atmospheric double drum drier. Steam was introduced into the drums under 75 lb. pressure. The cabbage was in contact with the drums about 30 seconds and was automatically scraped off as nearly dry flakes. This material was at once transferred to covered containers in contact with solid CO_2 and kept over night, when it was stored in flasks and evacuated.

2. Northern New York winter cabbage (batch 21) was steamed for 35 minutes and 50% of its weight was removed as press juice. The leaves were then separated and put in trays in a current of air at room temperature. Drying was completed in about 36 hours and the product represented from 7 to 9% of the original whole steamed cabbage.

Both of these dry preparations were moistened with water before feeding and given to rabbits in proportion to body weight. The rabbits ate it readily, gained weight and maintained a sleek healthy

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1 Marine, D., Baumann, E. J., and Cipra, A., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 822.

appearance. Control rabbits were fed with steamed pressed cabbage cake from the same lot of cabbage without drying.

The principal data are given in Table I.

TABLE I.

Sex	Weight at Beginning	Preparation Number	Amount Fed per day gm.	No. days Fed	Weight at End	Condition of Thyroid
M	1200	1	41*	26	1365	Normal
F	1128	1	38*	26	1227	Normal
M	2260	2	53*	21	2206	Normal
F	2300	2	53*	21	2351	Normal
F	1845	Control	214* Pressed cake	18	1703	Moderate hyperplasia
F	2290	Control	266* Pressed cake	18	2175	Moderate hyperplasia

* Supplementary diet of 17 gm. oats and 20 gm. alfalfa hay twice weekly.

These experiments indicate that cabbage loses its goitrogenic quality when dried by the methods indicated.

5096

Effect of Acid and Alkaline Hydrolysis on the Goitrogenic Substance Contained in Cabbage.

BRUCE WEBSTER* AND ANNA CIPRA.

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Further studies have been carried out in an effort to determine the nature of the goiter-producing substance contained in cabbage and other vegetables.[1, 2]

It was first considered advisable to attempt to find out whether or not this goiter-producing factor was soluble in water. Cabbage was steamed for 30 minutes, hashed and pressed until 50% of the total weight had been removed. The residue thus obtained had previously been shown[2] to contain a considerable proportion of the goiter-producing substance. Samples of this residue were washed with one and two volumes of water respectively, at room temperature and the water removed by pressing. As shown in the accom-

* Fellow of the National Research Council.

[1] Webster, Bruce, and Chesney, A. M., *Am. J. Path.*, 1930, vi, 275.

[2] Marine, D., Baumann, E. J., and Cipra, Anna, PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 822.

panying table, no marked change in the goiter-producing power occurred in either instance. Similarly, boiling the above residue for 15 minutes in 2 volumes of water, and pressing to its original weight did not materially alter its goitrogenic power.

Studies were then made of the effect on the goitrogenic power of cabbage of extracting with water at various hydrogen ion concentrations. The fraction which remained, after removal of 50% of the total weight as juice, from steamed hashed cabbage, was allowed to stand for 15 minutes at room temperature in 2 volumes of water to which sufficient hydrochloric acid had been added to bring the pH of the mixture to 3.0. The water was then pressed

TABLE I.

Rabbit No. and Sex		Cabbage Lot No.	Method of Treatment of Cabbage Cake	Duration of Feeding in Days	Condition of Thyroid Gland at Termination
847	F	21	Extracted for 15 minutes	23	Moderate hyperplasia
848	F	21	at room temperature with 2 vol. water. Pressed to original weight.	23	" "
865	M	21	Boiled for 15 minutes	21	" "
866	F	21	with 2 vol. water. Pressed	21	" "
863	M	22	to original weight.	21	" "
902	M	22	"	21	" "
759	F	9	Extracted with 2 vol.	18	Marked "
791	F	9	water at pH 3.0 at room	23	" "
826	M	9	temp. for 15 minutes. Pressed to original wgt.	23	Moderate "
849	F	21	Boiled with 2 vol. water	23	Marked "
850	F	21	at pH 3.0 for 15 minutes.	23	" "
869	M	21	Pressed to original wgt.	21	" "
870	F	21	"	21	" "
847	F	23	"	19	" "
903	M	23	"	19	Moderate "
871	M	21	Boiled with 2 vol. water	21	Marked "
872	F	21	at pH 3.0 for 30 minutes. Pressed to original wgt.	21	" "
873	M	21	Boiled with 2 vol. water	21	Moderate "
874	F	21	at pH 3.0 for 60 minutes.	21	
773	M	21	Pressed to original wgt.	21	" "
885	M	21	"	21	" "
851	F	21	Extracted with 2 vol.	23	Slight "
852	F	21	water at pH 9.0 at room temp. for 15 minutes. Pressed to original wgt.	23	" "
867	M	21	Boiled with 2 vol. water	21	Moderate "
868	F	21	pH 9.0 for 15 minutes. Pressed to original wgt.	21	Slight "
853	F	21	Control. Untreated cab-	21	Moderate "
854	F	21	bage cake.	21	" "
861	M	22	"	21	" "
901	M	22	"	21	Slight "

All animals in this experiment were given a supplementary diet of 17 gm. oats and 20 gm. alfalfa hay twice weekly.

out and the residue fed to rabbits. Such a procedure invariably produced a definite increase in the goiter-producing power of the cabbage. Boiling the cabbage in 2 volumes of water at pH 3.0 for 15 minutes further increased its goitrogenic power. Boiling at pH 3.0 for 30 minutes gave no detectable improvement over boiling for 15 minutes. Boiling for one hour, on the other hand, tended to decrease, but not to destroy entirely, its ability to produce thyroid hyperplasia. The results of the above experiments are summarized in the accompanying table. The feeding of equivalent amounts of hydrochloric acid to control animals did not produce goiter.

Attempts were made to study the effect of extracting the cabbage at pH 9.0. Since it has been shown[3] that the feeding of excessive amounts of alkali will, in itself, produce thyroid hyperplasia in animals, the results of our experiment are difficult to interpret. However, it appeared that extraction of the cabbage at pH 9.0 tended to lessen its goitrogenic power.

The results of these experiments would suggest that the goiter-producing substance contained in cabbage and other vegetables is not readily soluble in water, either at room temperature or at 100°C. Further, mild acid hydrolysis does not extract this substance. Prolonged acid hydrolysis at high temperatures may extract or destroy it to a slight degree. Alkaline hydrolysis appears to extract or destroy the goiter-producing factor.

The apparent increase in goitrogenic activity after contact with acid, may be due to a preservation of the goitrogenic substance, or on the other hand, it may be due to the destruction or extraction of anti-goitrogenic agents.[4] Further experiments along these lines are in progress.

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[3] Marine, D., unpublished data.

[4] Marine, D., Baumann, E. J., and Webster, B., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 1029.

5097

Occurrence of Antigoitrogenic Substances in Plant Juices.

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The discovery by Chesney, Clawson and Webster[1, 2, 3] that cabbage when fed to rabbits as their principal food produces marked thyroid hyperplasia has provided a rapid and more practical method for testing various antigoitrogenic agents. Last year we showed that cabbage grown in the spring and early summer is much less goitrogenic than cabbage maturing in the late autumn.[4] Also it was pointed out that whole press juice and juice concentrates made from potent goiter-producing cabbage had little or no goitrogenic activity.

Further work showed that the goitrogenic activity of cabbage was in general inversely proportional to its ability to absorb iodine. This suggested that there were 2 substances in cabbage in variable amounts, one of which was goitrogenic and the other antigoitrogenic. This view is further supported by the fact that washing hashed, steamed cabbage with water increases its goitrogenic activity. Since the antigoitrogenic activity corresponds roughly to the amount of reducing substance (determined iodometrically), we have sought an available plant which contained the reducing substance in greater amounts than is ordinarily found in common vegetables. We have obtained very potent concentrates from the juice of skunk cabbage *(Symplocarpus foetidus)* and have found that it produces thyroid involution in rabbits in from 8 to 10 days when fed by mouth daily in amounts equivalent to 100 units (a unit being the amount of juice required to absorb one cc. of N/100 iodine) in addition to our standard stock diet of oats and alfalfa hay. Sterile fractions introduced intraperitoneally appear to be more effective. Fresh cut alfalfa and lawn grass also produce involution of thyroid hyperplasia when fed to rabbits in amounts of 300 to 400 gm. daily for 3 weeks. The early spring growth of all of these plants contains large amounts of the iodine-absorbing material. Plant juices which are extremely low in the reducing substance have little or no

* Fellow of the National Research Council.

1 Chesney, A. M., Clawson, T. A., and Webster, B., *Johns Hopkins Hosp. Bull.*, 1928, xliii, 261.

2 Webster, B., Clawson, T. A., and Chesney, A. M., *Johns Hopkins Hosp. Bull.*, 1928, xliii, 278.

3 Webster, B., and Chesney, A. M., *Johns Hopkins Hosp. Bull.*, 1928, xliii, 291.

4 Marine, D., Baumann, E. J., and Cipra, A., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 822.

antigoitrogenic action. This antigoitrogenic substance is present in a great variety of plants but in highly variable amounts as regards age, species and climate.

Whether this reducing substance is identical with that which Szent-Györgyi[5] isolated from cabbage, orange juice and suprarenal cortex and found to be a hexuronic acid has not been established. All the evidence at present indicates that this reducing substance is at least one of the substances causing thyroid involution. The facts that a similar reducing substance occurs in the suprarenal cortex, the corpus luteum and the gonads and that we[6] have demonstrated that extracts of the suprarenal cortex have an inhibiting or regulatory effect on thyroid activity also support the view that the reducing substances in plants which involute thyroid hyperplasia belong to the same group of compounds which Szent-Györgyi isolated from the suprarenal. There is also evidence that this reducing substance hastens thymus involution and increases the weight of the suprarenals in rabbits. The involuting effect of these reducing substances is not due to iodine.

The iodine content of sheep, beef and hog thyroids as shown by Seidell and Fenger[7] is lowest in the early spring months. On the other hand the incidence of goiter in animals is highest during this season of the year when the amount of reducing substances in stored foods is at its lowest level. As the animals obtain fresh green food, the iodine content of the thyroid rises. Up to the present this rise in the iodine store has been assumed to be dependent upon an increased intake of iodine, but from the observations we have made it appears probable that the iodine store rises because the thyroid hormone is less needed. The relation of the suprarenal glands and gonads to thyroid enlargement also becomes more understandable in the light of these experiments. The suprarenals and gonads are normally a storehouse and possibly a place of manufacture of the reducing substance and so long as the supply of reducing substance is available in ample quantities it exerts a sparing action on the thyroid. We have repeatedly pointed out, in discussing the relation of iodine to thyroid enlargement,[8] that one must recognize those enlargements dependent upon an absolute deficiency of iodine (endemic goiter) and those dependent upon a relative deficiency (sporadic goiter), that is, due to conditions which increase the needs of the body for iodine as occurs in the thyroid enlargement of puberty,

[5] Szent-Györgyi, A., *Biochem. J.*, 1928, xxii, 1386.

[6] Marine, D., Baumann, E. J., and Cipra, A., *Am. J. Physiol.*, 1925, lxxii, 248.

[7] Seidell, A., and Fenger, F., *J. Biol. Chem.*, 1913, xiii, 517.

[8] Marine, D., *Medicine*, 1927, vi, 127.

pregnancy, Graves' disease and other associations. One of the conditions producing the relative iodine insufficiency now appears to be a deficiency in the reducing substance.

It is our present belief that plant juices prevent or cure thyroid hyperplasia by a thyroid-sparing acting, that is, by providing another mechanism for promoting tissue oxidations. On the other hand, iodine administration prevents thyroid hyperplasia by making it easier for the thyroid to produce more thyroxin.

5098

Action of X-rays on Glutathione Content and Oxygen Consumption of Normal and Regenerating Planarians.*

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Studies upon regeneration[1] have been carried out to test the hypothesis that persistent embryonic cells are responsible for the formation of new tissues during regeneration, and that the power or capacity for regeneration is to be correlated with the number of such cells present. It has been shown that in different species of planaria, cells of an embryonic type, called "formative cells," undergo rapid proliferation after cutting the planarian, migrate to injured areas, and differentiate into the new tissues characteristic of the regenerating part. Species of planarians that exhibit the greatest capacities for regeneration possess the greatest numbers of these formative cells. Hickman[2] destroyed the capacity for regeneration by the use of X-rays and radium, and histological studies of such irradiated worms showed a selective destruction of these formative cells.

Other investigators showed that the rate of metabolism and of oxygen consumption in particular, increase immediately after cutting and remain at a high level throughout regeneration. The important rôle of glutathione in the establishment of the metabolic level and in the utilization of oxygen indicated that important cor-

* Acknowledgements are due the Committee on Effects of Radiation, National Research Council, for support received through W. C. Curtis.

1 Curtis, W. C., *Proc. Boston Soc. Nat. Hist.*, 1902, xxx, 515.

2 Curtis, W. C., and Hickman, J. F., *Anat. Rec.*, 1926, xxxiv, 145.

relations might be found between the processes of regenerative development and the quantitative distribution of this compound. Numerous workers have found glutathione in greater concentrations in the tissues of embryos and in adult tissues capable of continued cell division than in adult tissues in general. Recently Hammett[3] has shown glutathione to be a stimulus to cell division.

Using a modification of methods of Joyet-Lavergne[4] and Hammett[3] the glutathione content was determined in two species of Turbellarians, *Planaria agilis,* and *Procotyla fluviatilis (Dendrocoelum lacteum).* The former species possesses a high capacity for regeneration and shows a high concentration of glutathione; the latter, which exhibits a low capacity for regeneration, shows a lower content. In normal worms the formative cells are found in the parenchyma, principally in the dorsal region. This area corresponds to that showing the highest content of glutathione. In regenerating individuals greater numbers of differentiating formative cells and higher glutathione content are found in the regenerating areas that form the head, tail, and pharynx.

Quantitative examinations of the glutathione content of normal, regenerating, and irradiated specimens of *Planaria agilis* were made by colorimetric estimations and by the Tunnicliffe[5] method. The glutathione content was found to decrease after irradiation with heavy exposures of X-rays (approximately 2,000 R units). Some typical results are found in Table I.

TABLE I.
Glutathione Content of Planarians Before and After X-ray Treatment (in mg. per 100 gm. of tissue).

Animals	Normals	2 hrs. after Irradiation
Pure line R-1-3	73	65
" " R-2-4	86	53
" " R-1-4	79	66
Mixed culture	94	81
Mixed culture	94	81
Average	83	65.75

Determination by Tunnicliffe method.

The oxygen consumption of individual specimens of *Planaria agilis* of the above types has been determined by the Winkler method. A micro-adaptation was devised in which determinations were made on small samples of fluid in an atmosphere of pure hydrogen. The

[3] Hammett, F. S., *Protoplasma,* 1919, vii, 297.

[4] Joyet-Lavergne, Ph., *C. R. Soc. de Biol.,* 1928, xcviii, 658.

[5] Tunnicliffe, H. E., *Biochem. J.,* 1925, xix, 194.

results upon regenerating worms confirm those of other workers. Oxygen consumption remains above normal during regeneration. Oxygen consumption of both normal and regenerating worms is markedly decreased by heavy exposures with X-rays. The profound effect of this treatment on the oxygen consumption may be seen from Table II.

TABLE II.

Oxygen Consumption (cc. per hr.) of Individual Planarians Before and After X-ray Treatment.

Animals	Normals before irradiation	24 hrs. after irradiation
A	0.01472	0.00114
B	0.00924	0.00027
C	0.00876	0.00110
D	0.00559	0.00037
E	0.01186	0.00570

Determinations by a micro-modification of the Winkler method.

In view of the stimulating action of glutathione upon cell division and the high concentration of this compound in embryonic cells, the destruction of glutathione by X-rays or at least its inactivation offers an explanation of the selective action of X-rays upon cells undergoing mitotic division and upon embryonic cells of planarians and other forms.

5099

Action of Adrenalin on the Metabolism of Peripheral Tissues.

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From the Department of Physiology, University of Buffalo Medical School.

It has been found impossible to affect the carbon dioxide or total acid production of excised frog muscles by placing them in adrenalin solutions.[1] The absence of a calorigenic effect[2] under such unphysiological conditions was not surprising; and more recent work has indicated that the absence of the viscera[3] and particularly of the liver[4] may have been as responsible for the negative result as the

1 Griffith, *Am. J. Physiol.*, 1923, lxv, 15.

2 Boothby and Sandiford, *Am. J. Physiol.*, 1923, lxvi, 93.

3 Soskin, *Am. J. Physiol.*, 1927, lxxxiii, 162.

4 Casky, *Am. J. Physiol.*, 1927, lxxx, 381.

lack of proper circulation or other abnormal conditions to which the muscles themselves were immediately exposed.

It is not known in what manner the viscera cooperate in the calorigenic action of adrenalin; whether by serving as the locus of its action, or by keeping the peripheral tissues in the proper physiological condition to respond to the adrenalin itself. The following experiments were undertaken to determine some of the effects that might be produced in one of the hind legs of an anesthetized cat upon the addition of a known amount of adrenalin directly to its arterial blood supply.

All experiments were done on cats under chloralose anesthesia. The animal was always prepared so that simultaneous samples could be taken of the arterial blood going to and venous blood coming from the left hind leg. At the time of collection of the venous sample the rate of flow was also determined in a manner somewhat similar to that described by Himwich and Castle.[5] The usual precautions were taken to prevent alteration in the gas content of the blood; and this was determined by the manometric method of Van Slyke and Neill, using 0.2 cc. samples in duplicate.

In large cats that could stand the additional loss of blood, 2 normal sets of samples, 10 or 15 minutes apart, were taken before the adrenalin injection; these served to establish the average spontaneous variation to be expected under the conditions of these experiments. In the remaining experiments the adrenalin was injected immediately after taking a single normal pair of samples.

The adrenalin was made up in 0.9% NaCl or mammalian Ringer from 1:1,000 Parke-Davis adrenalin chloride solution, neutralized and warmed before injection; and this was made directly into the iliac artery so that the adrenalin acted first, and we believe, in most cases, only on the tissues of the leg under observation.

Results: As controls we have 18 pairs of normal samples taken 10-15 minutes apart before injecting adrenalin; the average of these gives for the rate of blood flow and oxygen consumption of the first and second samples, respectively, 30.9 and 1.77; and 30.1 and 1.74 (all are cc. per minute). In other words in the 10 to 15 minutes elapsing between the first and second blood samples the rate of blood flow decreased only 0.8 cc. per minute and the oxygen utilization only 0.03 cc. per minute.

The effect of injecting adrenalin at the rate of 0.0004 mg. per cc. per minute for 5 minutes; we give the average of 10 experiments; in these the rates of blood flow and of oxygen consumption just

[5] Himwich and Castle, *Am. J. Physiol.*, 1927, lxxxiii, 92.

before the injection were, in cc. per min., 29.0 and 2.05; just after the injection they were, 26.0 and 1.93, *i. e.,* the adrenalin decreased the blood flow 3.0 cc. and the oxygen utilization 0.12 cc. per minute. We have another set of 9 experiments in which the adrenalin was given at just twice the rate used above, *i. e.,* 0.0008 mg. per cc. per minute for 5 minutes; the average rate of flow and of oxygen consumption just before these injections was 33.8 and 2.44, respectively; just after, the figures are, 27.6 and 2.14; *i. e.,* this dose of adrenalin slowed the rate of blood flow by 6.2 cc. per minute and the oxygen utilization 0.30 cc. per minute.

Although these decreases of oxygen utilization are not large they are 4 and 10 times greater than the change observed to occur spontaneously during an interval 2 or 3 times as long between normal samples; but even more significant, perhaps, is their constancy. Of the 18 pairs of normal samples, the oxygen consumption of the second was less than the first in 11 and greater in 7; of the 19 adrenalin experiments the oxygen consumption following the injection was less than the normal in 14, unchanged in 1, and greater in only 4.

These figures are derived from uncorrected oxygen contents; the determination of the oxygen capacity of 33 pairs of arterial-venous pairs gave an average venous capacity 0.46 vol. %, less than the arterial; this is not only opposite in sign to what has previously been reported (see 5) but also cannot be due to changes in cell volume, which, as an average of 27 determinations, was 55.07 for arterial, and 54.98 for the venous samples.

The effect of adrenalin on the carbon dioxide production was too variable and uncertain to justify speaking of it until further work is done.

5100

Dynamics of Insulin Secretion by the Pancreas and Epinephrine Secretion by the Suprarenal Gland.*

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Many authors have tried to prove that the pancreatico-duodenal vein is the carrier of the pancreatic hormone but nobody was able

* This paper has been partly supported by the Committee for Chemistrysation of SSSR, Moscow.

to accomplish this for two reasons: (1) Blood was taken in artificial conditions of acute experiment;[1] (2) for detection of insulin no direct biological, but only unspecific and indecisive indirect tests[2] were used.

We obtained the blood from the pancreatico-duodenal vein of healthy dogs under quite normal conditions by means of angiostomical tubes.[3] For detection of insulin we used the most sensitive biological test of Brugsch and Horsters[4]—hypoglycemic reaction of blood accompanied sometimes by paresis or paralysis of the legs of fasting white mice after intraperitoneal injection of the analyzed blood.

In our experiments the blood drawn from the pancreatico-duodenal vein of fasting angiostomised dogs before and after intravenous injection of 25-50 gr. of glucose was introduced in amounts from 0.2 to 1.0 cc. into the peritoneal cavity of white mice. Two to 3 hours after injection the mice were killed by decapitation and their blood sugar evaluated by the method of Hagedorn-Jensen. The results show in a definite way that in a fasting dog insulin cannot be detected in the pancreatico-duodenal blood even with very sensitive biological tests. After intravenous injections of glucose considerable amounts of insulin are present in the blood of the pancreatico-duodenal vein. There was in literature an uncertainty whether insulin is secreted in an active or in an inactive state; our experiments prove that it is secreted into the bloodstream in an active state.

Contrary to the insulin secretion of the pancreas the epinephrine secretion by the suprarenal glands seems to proceed continually in a fasting dog. We have successfully applied the Brugsch insulin test in our experiments for the detection of epinephrine in blood, hyperglycemic reaction resulting instead of the hypoglycemic one.

The hyperglycemic effect of the blood of the lumbalo-suprarenal vein of a fasting dog injected into the peritoneal cavity of fasting white mice proved to be very characteristic. After injection into the peritoneal cavity of white mice of the same amounts (1 cc.) of the blood taken from the femoral vein of the same dog there could be found no rise of the blood-sugar level.

The amounts of insulin contained in the pancreatic vein after injection of sugar amounts to approximately 0.01 unit per 100 cc., whereas the amount of epinephrine found in the lumbalo-suprarenal vein amounts to 0.00001%.

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[1] La Barre, *C. R. Soc. Biol.*, 1929, ci, 144; 1927, xcvii, 1801.

[2] Diedrich, *Arch. exp. Path. und Pharm.*, 1922, cxxv, 336.

[3] London, E. S., *Harvey Lectures*, 1927-28, 288.

[4] Brugsch und Horsters, *Arch. exp. Path. und Pharm.*, 1930, cxlviii, 295.

5101

Effect and Mode of Action of Tartrates in the Human Body.

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Though tartrates have long been used in medicine, and grapes and grape products, the chief sources of tartrates are common foods, there is still much difference of opinion as to the effect and mode of action of tartrates in the human body. Among the unsettled questions are: 1. Are tartrates absorbed from the intestine? 2. If absorbed, are they oxidized in the tissues? 3. Are they alkalinizing? 4. If alkalinizing, how is this effect produced? Solis-Cohen and Githens,[1] Blatherwick,[2] Post,[3] and others believe tartrates are alkalinizing, the basis of their belief evidently being the idea of absorption and oxidation. Pickens and Hetler,[4] Wood,[5] Simpson,[6] Rose,[7] and others are on the contrary side of the alkalinizing question. Sollmann,[8] McGuigan[9] and others take a middle ground.

We can not tell whether tartrates are absorbed from the human intestine or not; but if they were absorbed in the case of any of our experimental subjects they were evidently oxidized, for we found no tartrates in the urine after their ingestion in quantities as great as 2 U. S. P. doses in a day's intake.

Rochelle salts, grapes, raisins and grape juice all proved to be definitely alkalinizing. Any of these substances, when added to an acid-forming diet, markedly decreased urinary acidity.

This alkalinizing effect of tartrates would be strong evidence of their absorption and oxidation, were it not for another process that explains this effect. In mixtures of feces and Rochelle salts, incubated at 37.5°C., the tartrate ions were decomposed within 6 to 24 hours. This proved true without exception in 15 cases. Bile and

1 Solis-Cohen, S., and Githens, T. S., "Pharmacotherapeutics, Materia Medica and Drug Action," 1928, 1175 and 1176.

2 Blatherwick, N. R., *Arch. Int. Med.*, 1914, xiv, 409.

3 Post, W. E., *J. Am. Med. Assn.*, 1914, lxii, 592.

4 Pickens, L. M., and Hetler, R. A., *J. Home Econ.*, 1930, xxii, 44.

5 Wood, H. C., LaWall, C. H., and others, *The Dispensatory of the United States of America*, 21st ed., 1926, 878.

6 Simpson, G. E., *J. Pharm. and Exp. Ther.*, 1925, xxv, 459.

7 Rose, W. C., *J. Pharm. and Exp. Ther.*, 1924, xxiv, 123 and 147.

8 Sollmann, T., "A Manual of Pharmacology," 1928, 47.

9 McGuigan, H. A., "A Textbook of Pharmacology and Therapeutics," 1928, 282.

pancreatin seem unable to decompose tartrate ions. Berkefeld filtrates of feces have this ability to a very limited extent, if at all. Carefully controlled tests with *Escherichia coli,* communior and acidi-lactici, however, have shown that all of these organisms can decompose the tartrate ions in Rochelle salts and produce alkali from this compound by this means.

The alkalinizing effect of tartrates, caused by bacterial decomposition of tartrate ions in the lumen of the intestine and the consequent freeing of easily-absorbable sodium and potassium ions, is probably their chief mode of action in cases where catharsis is not produced.

Our work is still in progress, and more detailed reports are in process of preparation.

5102

A New Synthesis of Aspartic Acid.*

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In previous syntheses of aspartic acid the following reactions have been used: The decomposition of acid ammonium malate by heat,[1] the racemization of active aspartic acid[2] and active asparagine,[3] the reaction of maleic or fumaric acid with ammonia,[4] the reduction of oxalacetic ester oxime,[5] the reaction of silver fumarate or potassium acid fumarate with hydroxylamine hydrochloride,[6] the reduction of nitrosuccinic ester,[7] the catalytic reduction and ammonation of oxalacetic acid,[8] and the hydrolysis of the addition compound formed from sodium malonic ester and chloroacetic ester.[9]

*Financial assistance in this work has been received from the research funds of the University of California.

[1] Dessaignes, *Compt. rend.*, 1850, xxx, 324; *Ibid.*, 1850, xxxi, 432. Wolff, *Ann.*, 1850, lxxv, 293.

[2] Michael and Wing, *Ber.*, 1884, xvii, 2984; *Am. Chem. J.*, 1885, vii, 278.

[3] Piutti, *Ber.*, 1886, xix, 1691.

[4] Engel, *Compt. rend.*, 1887, civ, 1805; *Ibid.*, 1888, cvi, 1734; Stadnikoff, *Ber.*, 1910, xliv, 44.

[5] Piutti, *Gaz. Chim. Ital.*, 1887, xvii, 519.

[6] Tanatar, *Ber.*, 1896, xxix, 1477.

[7] Schmidt and Widman, *Ber.*, 1909, xlii, 497.

[8] Knoop and Oesterlin, *Z. physiol. Chem.*, 1925, cxlviii, 294.

[9] Keimatsu and Kato, *J. Pharm. Soc. Japan*, 1929, xlix, 111 and 123.

The present synthesis is believed to be more satisfactory than those previously reported for aspartic acid.

Phthalimido malonic ester, prepared from potassium phthalimide and bromodiethylmalonate, was converted to sodium phthalimido malonic ester by allowing it to react with molten metallic sodium suspended in toluene. A stable addition product was then formed as a dark colored oil by the reaction of chloroacetic ester with sodium phthalimido malonic ester. This product was readily hydrolyzed by hydrochloric acid with the formation of d,l aspartic acid, phthalic acid, carbon dioxide, and ethyl alcohol. Side reaction products are not probable.

The acid hydrolysate was evaporated to precipitate the phthalic acid which was removed by filtration. Hydrochloric acid was removed by the addition of the calculated quantity of silver oxide to the boiling diluted filtrate. After removing the precipitate of silver chloride and evaporating the filtrate to a small volume d,l aspartic acid crystals separated after standing for 3 days. Upon recrystallization from hot, 50% ethyl alcohol these crystals darkened and decomposed over the range, 311-325°C. Within the limits of experimental error they were shown to contain the theoretical quantity of amino nitrogen.

Additional experiments on the reaction of ethylene bromide, ethylene chloride, methylene bromide, beta chloropropionitrile, beta chloropropionic ester, and trimethylene bromide were carried out. In all cases the liberation of halide ions was nearly complete, but only with trimethylene bromide was the expected addition product formed. This reaction has been used by Sorenson[10] for the synthesis of proline.

5103

Use of Phenol Red in the Addis Test of Renal Function.

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Under certain special conditions the rate of urea excretion in the urine is directly proportional to the blood urea concentration so that the ratio $\frac{\text{Urine rate}}{\text{Plasma concentration}}$ becomes a constant.[1] Under these

[10] Sorenson, *Z. physiol. Chem.*, 1908, lvi, 236.

[1] Addis, T., and Drury, D. R., *J. Biol. Chem.*, 1923, lv, 105.

conditions this ratio varies only with the amount of functioning renal tissue[2, 3] and can be used as an accurate method for measuring renal function in man.[4] It has recently been shown[5] in the

TABLE I.

Time of Urine Collection	Urine volume cc./hr.	Excretion		Blood sample	Plasma Concentration		Urine rate / Plasma concentration Ratio:	
		Phenol red mg./hr.	Urea mg./hr.		Phenol red mg./100cc.	Urea mg./100cc.	Phenol red	Urea
11:00-11:20	620	858	1737	11:10	5.80	36.2	149	48
11:20-11:40	300	599	1460	11:30	3.90	28.5	153	51
11:40-12:00	750	360	1366	11:50	3.25	26.2	160	52

[2] Taylor, F. B., Drury, D. R., and Addis, T., *Am. J. Physiol.*, 1923, lxv, 55.
[3] Addis, T., Myers, B. A., and Oliver, J., *Arch. Int. Med.*, 1924, xxxiv, 243.
[4] Addis, T., *Arch. Int. Med.*, 1922, xxx, 378.
[5] MacKay, E. M., and Oliver, J., *J. Exp. Med.*, 1930, li, 161.

rabbit that phenol red is excreted in a manner similar to urea in so far as the relation of the urine rate to the plasma concentration is concerned. This has led us to examine the possibility of using phenol red in place of urea in performing the Addis test of renal function[4] which has been referred to. The results presented in Table I which were obtained from a subject without renal disease after the injection of 1 gm. phenol red* during an urea diuresis indicate that it may be feasible to use phenol red in place of urea to determine the Addis excretory ratio. The difference between the urea and phenol red ratios which is apparently constant will be discussed elsewhere. There are certain advantages over urea in the use of the dye, particularly in the determinations in the plasma and urine. The actual concentrations need not be measured. After appropriate dilution and alkalinization the serum and plasma are compared in the 2 cups of a good colorimeter and the ratio calculated from the readings and respective dilutions. The clinical application of the use of phenol red in this manner is being investigated.

5104

Inactivation of Staphylococcus Bacteriophage by Toluidine Blue.

C. E. CLIFTON AND T. G. LAWLER. (Introduced by E. W. Schultz.)

From the Department of Bacteriology and Experimental Pathology, Stanford University, California.

The inactivation of staphylococcus bacteriophage by methylene blue was reported by Schultz and Krueger.[1] Further tests in this laboratory with various samples of methylene blue* have given practically the same results, concentrations of 0.05% of the dye inactivating the bacteriophage within 24 hours. In higher dilutions longer periods of time were required for inactivation, whether the tests were carried out at room temperature or at 37°C. In only a few isolated cases was an inactivation obtained with concentra-

* We are indebted to the firm of Hynson, Westcott and Dunning of Baltimore for making this study possible through their very generous cooperation in supplying strong phenol red solutions suitable for intravenous use.

[1] Schultz, E. W., and Krueger, A. P., PROC. SOC. EXP. BIOL. AND MED., 1928, xxvi, 101.

* Merck's U. S. P. Methylene Blue (3 samples); Coleman and Bell, U. S. P. Methylene Blue; National U. S. P. Methylene Blue (2 samples); Grubler's Methylene Blue.

tions below 0.005%, when the ordinary bacteriophage suspensions were used or when such suspensions were diluted 1-10 with Martin's broth.

Similar tests have been carried out with various other dyes including toluidine blue, methylene violet, methylene green, methylene azur, thionin, eosin B, and phenol red. These dyes were added in relatively high concentrations to bacteriophage filtrates which were then incubated at 37°C. for 24 hours or longer and tested for lytic activity. Of the series tested in this way only one, *toluidine blue,* served to inactivate the staphylococcus bacteriophage.

Several individual samples of the same brand of toluidine blue† have given results which in all respects were similar to those obtained with methylene blue. When incompletely inactivated dye-phage mixtures were plated out with susceptible organisms a diminution in the number of plaques was noted, both in the case of methylene blue and of toluidine blue.

Tests were made with the dye-phage mixtures which were incubated in an atmosphere of hydrogen, nitrogen, or oxygen. The results obtained were identical with those described above, while controls incubated under the same conditions retained their lytic activity.

Inactivation was not obtained with either methylene blue or toluidine blue when added to an anti-coli bacteriophage. The results, therefore, indicate that the inactivation is a specific phenomenon, affecting particularly the staphylococcus bacteriophage. Filtration studies suggest that the inactivation is probably due to an adsorption phenomenon.

5105

The Hearts of Wild Animals in Captivity.

GEORGE HERRMANN.

From the Department of Pathology of the Audubon Zoo and the Department of Medicine of Tulane University of Louisiana, New Orleans.

Comparative anatomical studies of the heart in various mammals, with reference especially to the relation of the heart weight to the body weight, have yielded much interesting information. It was shown previously[1] that the heart of the racing greyhound averaged 13.8 gm. per kilo of body weight and stood at the top of the animal

† National Toluidine Blue (3 samples).

[1] Herrmann, George, PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 856.

series in respect to relative heart size. It was also found that even the young greyhounds who had been confined to small kennels had relatively large hearts. Similar conditions but less striking figures were found in the study of the heart of the thoroughbred race horse[2] in which the average of 8.65 gm. of heart per kilo of body weight would indicate a similar change. This enlargement of the heart was present also in the young horses, colts and yearlings. The seasoned racers in both series had relatively enormous hearts. It was suggested that there was perhaps an inheritance of this acquired characteristic in the enlarged heart which had the capacity for even greater enlargement under stress. The factor of selection in breeding, however, was considered probable as a most potent factor. The greyhound heart averaged 5 gm. per kilo higher than did the heart of the ordinary dog, 13.8 as contrasted to 7.98. The thoroughbred's heart averaged about 2 gm. more per kilo than the ordinary horse's heart, 8.76 as contrasted to 6.77.

Opportunity was afforded for pursuing comparative anatomical heart weight-body weight studies on the animals that had to be sacrificed or died in the zoo, and thus was added interesting information on the question of the relation of heart weight to activity from another point of view. The causes of death in these animals were those that do not affect heart weight. Most of the animals had been born in the wilds and had been in captivity since youth. A few were born in captivity. Younger animals as a rule, even though born in captivity had relatively larger hearts than the older animals. Increase in weight due to the sedentary life in the cages was probably conducive to abnormal body weight and thus disturbed the proportion. The heart of a baby stag red deer reached the high proportion of 15.4 gm. of heart per kilo of body weight, while in a full grown doe there were only 6.1 gm. of heart per kilo of body weight. The young male wolf was found to have 12.4 gm., while the female adult had 10.8 gm. of heart per kilo of body weight. The wolves thus averaged about 11.5 gm., which is 3 gm. more than the average for the police dog and 3.5 gm. more than the average for the ordinary dog. The sea lions averaged 7.56 gm., the lion cub of five months 7.11 gm., and an eleven months old lion, 4.77 gm. The leopard cubs averaged 6.5 gm. The wallabys averaged 7.05 gm., while a kangaroo was only 6.38 gm. per kilo. In the primate series the Sapajou monkeys averaged 6.43 gm. and about eight *Macacus rhesus* monkeys gave an average of 6.11 gm. The pig tailed monkeys 4.26 gm. and 2 green monkeys

[2] Herrmann, George, PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 549.

4.16; while the Japanese ape showed 5.0 gm. and a Sphinx baboon 4.05 gm. of heart per kilo of body weight. The squirrels averaged about 5.89 gm., the puma 4.63 gm., raccoon 4.22 gm., tapir 3.71 gm., and the sloths averaged 3.96 gm. The sloth thus takes the lowest position in the wild animal scale and the average is almost as low as that presented by the rabbit, which in the domesticated type is as low as 2.36 gm. per kilo of body weight. This series, incomplete though it is, tends to add weight to the idea that there is some fundamental relationship between the activity of the individual mammal and its heart weight, body weight ratio. The more sluggish the animal the relatively small the heart and the less the ratio. The size of the animal is perhaps a factor in some instances, in that in general in the large animals the body weight runs into hundreds of kilos and there is a tendency to have smaller hearts, in proportion to these great weights.

5106

Observations on the Effect of Dog's Gastric Juice in Pernicious Anemia.

L. T. COGGESHALL. (Introduced by W. L. Palmer.)

From the Department of Medicine, University of Chicago.

This experiment was undertaken to determine the effects of large amounts of dog's gastric juice on a patient suffering with pernicious anemia. Castle and Townsend[1] previously had shown that 300 cc. of human gastric juice administered daily for a 10-day period did not improve the patient symptomatically nor produce any change in the blood picture. Because the amount of gastric juice secreted each 24 hours by man is far in excess of the 300 cc. used, it was thought worth while to repeat the procedure with larger doses.

For this experimental work pure, crystal clear dog's gastric juice was used. This was procured in large quantities from Dragstedt[2] at whose suggestion the experiment was performed. He had obtained the juice in a previous work by creating a pouch of the entire stomach of the dog in such a way as to avoid injury of the fibers of the vagus or of the blood supply. The analysis of the product

[1] Castle and Townsend, *Am. J. Med. Sci.*, 1928, clxxviii, 693.

[2] Dragstedt, L. R., and Ellis, C. E., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 305.

showed a free HCl of 0.45%; total HCl, 0.5%; total chlorides, 0.55%, and pepsin 150 units.

The patient used in this experiment was Mr. S., Unit No. 18523, age 59, admitted to the Billings Hospital on January 20th, 1930, complaining of shortness of breath after slight exertion of 9 months' duration, numbness and tingling of his hands and feet, moderately severe nocturia and slight nausea following meals for a month preceding admission. These symptoms were associated with a progressive weakness during the past 5 months.

The physical examination revealed the following essential features: White male, apparently 65 years of age, with facial evidence of marked loss of weight, decided lemon-yellow tint of the skin and sclera and with only a slight impairment of the gait. There was a slight dyspnea on exertion but no cyanosis. The tongue margins were smooth with an atrophy of the central papillae. The chest was normal. The heart was not enlarged. There was a soft systolic "hemic" murmur heard at the apex and over the carotids. The systolic blood pressure was 140, the diastolic 80 mm. of mercury. The spleen and kidneys were not palpable. The prostate was firm and slightly nodular.

Neurologic examination showed all cranial nerves to be normal. The coordination power and tone of the muscles in the arms were normal. The vibratory sense was definitely decreased in the legs. The knee jerks were equal and normal. The sense of position, tactile and pain sense was normal in the legs. The Rhomberg was negative. The gait showed a slightly broadened base.

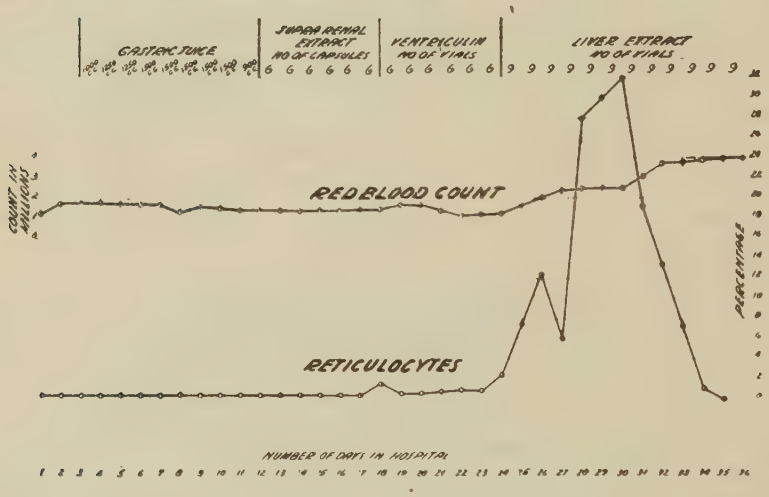
The blood picture showed the erythrocyte count to be 1,650,000, leucocytes 10,500 and Hb. 6.7 gms. per 100 cc. (Newcomer). The Price-Jones curve gave an average diameter of the red blood cell of 9.5 microns. The stained smear revealed numerous myelocytes and nucleated red blood cells, with a marked poikilocytosis and anisocytosis with a predominance of macrocytes. There was an achlorhydria as shown by the Ewald test meal, the alcohol test meal, and the response to the subcutaneous injection of 0.7 mg. of Histamine hydrochloride. The direct Van den Berg reaction was negative and the indirect was in the lower limits of normal. Repeated stool examinations were negative for gross and occult blood.

The patient was placed on a regular meat free diet and given 250 cc. of the dog's pure gastric juice 4 times daily. The dosage was given by mouth both as a drink and through a Rehfuss tube and in such relationship to meal periods as to produce the least amount of nausea possible. Nausea did occur but the patient man-

aged to retain the amounts prescribed. The dosage was increased to 350 cc. 4 times a day with the result that during the 10-day period he received approximately 12 liters of the pure gastric juice.

At the end of the interval the patient had not improved symptomatically but complained that the numbness and tingling of the hands had increased. Daily reticulocyte and erythrocyte counts failed to reveal any significant change. As is shown in Fig. 1 the hemoglobin remained constant, 6.7 gm. per 100 cc.

FIGURE NO. 1.
SHOWING PROGRESS OF PATIENT WITH PERNICIOUS ANEMIA TREATED WITH DOGS GASTRIC JUICE, SUPRA RENAL EXTRACT AND LIVER EXTRACT.



Following the treatment with the gastric juice, the patient was given an extract of the suprarenal gland (Koehler) in dosage of 6 capsules daily which corresponds to 30 gm. of the whole gland. The diet remained the same. At the end of 6 days no improvement was noted. It was later learned that this preparation of the extract had not been assayed.

The patient was then placed on Ventriculin, 6 vials daily, which was continued for 6 days. At the end of the interval there was a slight rise in the reticulocyte percentage. At this time we had had very little experience with Ventriculin, and the patient's condition was such that we did not feel justified in withholding liver any longer. He was immediately placed on liver extract and showed a prompt response. The reticulocyte count rose to 32% on the seventh day and the red blood count gradually increased until it was 4 million on

the twelfth day. At the time of discharge the patient still complained of slight tingling of the hands.

Conclusions. The above findings are in accord with the work of Castle and Townsend.[1] Clear dog's gastric juice in a total dosage of 12 liters given during a period of 9 days did not influence the reticulocyte or red cell count or give symptomatic relief in this case of pernicious anemia.

5107

A Study of the Effect of Insulin on Gastric Motility.

T. E. HEINZ AND W. L. PALMER.

From the Department of Medicine, University of Chicago.

In continuing the study of the mechanism of pain in gastric and duodenal ulcer a method for increasing the gastric motility was desired. Bulatoa and Carlson[1] showed that the injection of insulin into normal fasting dogs increased the motility of the stomach. Quigley, Johnson and Solomon[2] concluded from their investigations that the same held true for the stomach of normal fasting man. Clinicians have frequently observed hunger as a symptom of the so-called "insulin reaction". Hence it was assumed that the hunger so seen was due to the increased gastric motility, Carlson[3] having correlated "hunger pangs" with activity of the stomach.

This report is based on the results of 18 experiments done on 13 patients, 11 of whom had duodenal ulcer, 1 paroxysmal fibrillation, and 1 mild diabetes mellitus. The last 2 had a complete achylia to the histamine test. Kymographic records of gastric activity were made by means of the usual single balloon method connected to a water manometer, the entire system being inflated with 150 cc. of air. In a number of cases the position was checked by fluoroscopic examination. The tracings were all made with the balloon in the pyloric region of the stomach. Fasting periods ranged from 14 to 21 hours; the average time was 19 hours. The patients were instructed to register the appearance of "hunger pangs" by means of closing a switch set in series with a signal magnet.

In most instances control tracings had been obtained during previous experiments of a different nature. With those in which this

[1] Bulatoa, E., and Carlson, A. J., *Am. J. Physiol.*, 1924, lxix, 107.

[2] Quigley, J. P., Johnson, V., and Solomon, E. J., *Am. J. Physiol.*, 1929, xc, 89.

[3] Carlson, A. J., "Control of Hunger in Health and Disease," Chicago, 1916.

had not been done, a preliminary period was recorded before the administration of insulin.

Insulin (Mulford) was given subcutaneously in doses of 8 to 20 units. The period of observation after its injection varied from 1½ to 4 hours. Manifestations of insulin reaction were noted in all but 3 instances. The first symptom was usually that of a peculiar nervousness which gradually progressed. Upon this there soon developed a feeling of warmth and a gradually increasing desire for food, followed by profuse perspiration. Throughout the experiment the blood glucose level was followed, the determinations being made by the Folin method.

It seems to us important to differentiate the various factors involved in the phenomenon of hunger. Dr. Carlson has emphasized the difference between hunger and appetite. Appetite, of course, may be present with hunger. However, if one dissociates appetite from hunger there remain at least two factors which properly constitute the hunger complex, namely the epigastric "hunger pang", and a general weakness or desire for food. In some individuals the first dominates the picture of hunger, in others the second. In our work hunger, not appetite, but hunger in the sense of extreme desire for food, not associated with epigastric pain, was found to parallel quite closely the intensity of the insulin reaction. This general desire for food accompanied by weakness and nervousness was frequently present without definite gastric activity and, in 2 such instances especially it became so marked that the patients begged to be released. "Hunger pangs" wherever they occurred were correlated with hunger contractions, although there were occasional exceptions in which the pain was apparently due to activity lower down in the bowel.

The stomach in one patient was in a period of activity at the time of injection of insulin, C, which culminated in vigorous type iii contractions. The control tracings had shown exactly the same type of activity. During the subsequent 90 minutes there was practically no gastric motility, but after 105 minutes another hunger period apparently appeared. The blood sugar fell from 68 to 40 mgm. per 100 cc. and the patient experienced an intense desire for food.

In another patient, in the 2½ hours following the injection of insulin there was no gastric activity different from that seen in the control tracings in spite of the fact that the patient developed a marked insulin reaction. The blood sugar fell from 77 to 42 to 43 mgm. per 100 cc.

In another patient a tracing obtained with insulin on a previous occasion shows essentially the same reaction. There is seen a normal hunger contraction period ending in strong type iii contraction. In none of the control tracings for this individual was there such an extended rest period. Insulin was given at B which represents about one-half of the normal rest period. No definite activity was seen for 2 hours. Although the blood sugar was lowered from 63 to 43 to 47, no increased activity occurred. At the end of this experiment the patient was extremely weak, nervous, and had a marked desire for food.

In one instance we noted a definite prolongation of the contraction period. Insulin was given at B during a period of gastric activity. No rest period occurred during the entire experiment. At the end of 2½ hours the patient suddenly developed a severe insulin reaction which necessitated discontinuing the tracing.

Our studies thus far have failed with 2 exceptions to show any very definite or striking stimulation of gastric activity as the result of insulin. These 2 exceptions were cases in which there was increase in the length of the contraction period but not in the intensity of the contractions. Types ii and iii contractions were rather frequently seen without insulin, but in no case were they seen after the injection of insulin, even at the height of the reaction.

5108

Experimental Infarction of Bone Marrow.

ALEXANDER BRUNSCHWIG. (Introduced by George M. Curtis.)

From the Department of Surgery, University of Chicago.

In the course of a study of the etiology of solitary bone cysts, a series of experiments was performed, the object of which was to infarct large areas of bone marrow and to determine whether or not a cystic degeneration takes place in these areas with formation of lesions similar to the solitary bone cysts of man.

The animals used were young and adult dogs. Under ether anesthesia and careful aseptic precautions, the periosteum over nearly the entire femoral shaft between epiphyseal lines was elevated, thus severing all blood supply and drainage. Small areas of periosteum were left intact near the extremities of the shaft to afford a ready source of blood supply for revascularization of the infarcted areas.

Twenty-four hours following operation no change was noted.

Four animals sacrificed at various intervals from 4 to 8 days following operation show grossly infarction of the marrow of the shaft as evidenced by its pale dull yellow color in contrast with the red-brown appearance of normal marrow in the unoperated extremity. Near the epiphyseal lines are small sharply demarked red areas corresponding to the uninfarcted marrow beneath the small areas of intact periosteum. Microscopically, the marrow in the infarcted regions is necrotic, and the bony trabeculae well within the infarcted areas are dead. In the zone between infarcted and living marrow, there is, in places, a wide area of dense leucocytic infiltration, elsewhere the necrotic and living marrow are sharply demarcated without a zone of leucocytic infiltration.

A specimen studied 30 days after operation exhibited a porous cortex and red-brown normal appearing marrow in the metaphyses. In the upper portion of the shaft, the marrow cavity was filled by dense white fibrous tissue. Microscopically, this fibrous marrow is seen to vary considerably in density, and in degree of infiltration by leucocytes. In one region there was considerable new bone formation from the endosteal surface of the cortex. No cystic structures were present.

The femur of a case 60 days following operation exhibited considerable subperiosteal bone formation with normal marrow in the metaphyses but rather firm fibrous tissue in the central portion of the shaft, (*i. e.*, at greatest distance from metaphyseal arteries). Microscopically, the inner portion of the cortex of the shaft was seen to be dead and undergoing creeping substitution by new bone from the subperiosteal newly formed bone. The fibrous marrow is rather dense and is sharply demarcated from the adjacent fatty and cellular marrow. The outstanding feature, however, is that scattered throughout this region are numerous large and small rounded empty cavities, the walls of which are formed by condensation of fibrous tissues. There is little infiltration in the fibrous marrow and no foreign body giant cells. None of these cystic spaces bear any resemblance, histopathologically, to the solitary bone cysts of man.

Another specimen was studied 59 days following operation. This experiment was complicated by a healing fracture through the central portion of the shaft. Fibrous marrow without small cyst formation was present in the central portion of the shaft above and below the callus.

In 15 adult dogs with closed epiphyseal lines, no infarction was obtained by the above procedure. The reason for this no doubt is the adequate anastomoses between shaft and epiphyseal vessels.

The negative results obtained in the above series of experiments are reported because in the clinical literature on solitary bone cysts, trauma causing profound vascular disturbance, is repeatedly emphasized as an important etiologic factor.

Healing of sterile infarcted areas in the long bones of dogs does not result in lesions resembling solitary bone cysts in man. However, fibrosis of the marrow with multiple small cystic cavities lined by condensed fibrous tissue may develop.

5109

Is Sodium Salicylate Excreted in the Bile?*

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From the Departments of Pathology and of Surgery of the University of Chicago.

In the course of studies on the excretion of various chemicals by the liver, it was discovered that sodium salicylate and the sodium salt of diiodosalicylic acid did not appear in the bile of the rabbit in determinable quantities after the intravenous administration of 4 cc. of a 1% solution per kilo of body weight.[1] In view of the consequent discrepancy regarding the cholagogue and bactericidal action of salicylates on the biliary system, it seemed worth while to investigate the biliary excretion of salicylate in man. Three patients, 2 with drainage tubes in the common hepatic duct following cholecystectomy and choledochotomy, and one with a cholecystostomy, received by mouth a single dose of 20 mgm. of sodium salicylate per kilo of body weight. Samples of bile and urine were obtained before administration and after administration, namely 12, 40 and 79 hours respectively. The salicylate was determined colorimetrically by a modification of the Millon reaction.[2]

Studies made in one case: Housewife of 66, cholecystectomy for chronic cholecystitis and cholelthiasis, choledochotomy for stones in the common duct, and catheter in the common duct. The bile and urine were collected in hourly samples for 12 hours. The flow of bile averaged about 26 cc. per hour (with a minimum of 8 and a

* This work has been conducted under the joint auspices of the Otho S. A. Sprague Memorial Institute and the Douglas Smith Foundation for Medical Research.

[1] Halpert, Béla, and Hanke, Milton T., *Anat. Rec.*, 1929, xlii, 49.

[2] Folin, O., and Ciocalteu, V., *J. Biol. Chem.*, 1927, lxxiii, 627. Hanke, M. T., *J. Biol. Chem.*, 1928, lxxix, 587.

maximum of 48 cc.) and totaled 290 cc. for the 12 hours. Sodium salicylate could not be detected. The urine totaled 1381 cc. for the same period. This contained a total of 0.584 gm. of salicylic acid, corresponding to 0.680 gm. of sodium salicylate.

Sodium salicylate did not appear in the bile in recognizable amounts following the oral administration of doses of 20 mg. per kilo of body weight to the 3 patients with disease of the biliary system. These observations are in accord with data obtained in healthy rabbits.[1] The principal route for the elimination of sodium salicylate according to the above observations is the urinary tract. It is, therefore, reasonable to assume that whatever beneficial effect may follow the administration of this drug to patients with disease of the biliary system, is not due to the presence of salicylate in the bile.

5110

Further Observations on the Oxygen Consumption of Nerve.*

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The earlier studies of Fenn[1] and Gerard[2] on the oxygen consumption of frog nerve were not in complete quantitative agreement, even when differences in the experimental temperatures were allowed for. Fenn used American frogs *(pipiens?)* and a volumetric method, I used European species and a manometric technique, so it was desirable to ascertain whether the divergent results depended on material or methods. It also seemed useful to examine in more detail than previously the influence of various conditions on the rate of oxygen usage of nerve and to compare nerves of several animals. Observations carried on during 2 years by the Warburg technique are here summarized.

The Qo_2 (cmm. O_2 consumed per gm. of moist nerve per hour) of the sciatic of the American green frog, mainly *R. pipiens,* is lowest in December and January, $Qo_2 = 37$, and highest in June or a little earlier, $Qo_2 = 60+$. The Qo_2 shows a temperature coefficient of 2.2, as far as can be judged by experiments run at 18° to

* The present investigation was aided by a grant to the University of Chicago from the Rockefeller Foundation.

1 Fenn, *J. Gen. Physiol.*, 1927, x, 767.

2 Gerard, *Am. J. Physiol.*, 1927, lxxxii, 381.

24°C. The various American species do not consume oxygen at the same rate, but no accurate record of specific differences has been kept. The average Qo_2 of 227 experiments, mostly in the winter months, at an average temperature of 21.5°C. for green frog sciatics in Ringer solution is 42, and the extreme variation is 20 to 80. Fenn's[3] latest value for these same frogs at 20°C., is 45, so our results, with different methods, are in agreement.

Any influence of various substances on nerve respiration is dependent on their ability to penetrate to the respiring regions. The great importance of the perineurium as a passive barrier to diffusion, as described by Feng and Gerard[4] was not fully realized when most of these experiments were performed; and some of the negative results obtained may have depended on the presence of the sheath. Since, however, in most cases respiration remained constant for 10 to 20 hours, and since the few experiments on nerves with the sheath split longitudinally confirmed those on intact nerves, the present results may tentatively be considered valid.

The oxygen consumption is not influenced by the pH between 7 and 9. At pH 5.3 it is decreased about half. Borate buffer does not affect respiration, phosphates appear to cause an increase of 10 to 15%, independently of pH effects. Isotonic NaCl is equivalent to Ringer solution so far as oxygen consumption is concerned. 90% NaCl plus 10% of $CaCl_2$, KCl or $MgCl_2$, or plus 5% of any two of these is equivalent to 100% NaCl. Isotonic $CaCl_2$, KCl, or $MgCl_2$ may markedly decrease oxygen consumption to below 50%. Hypertonic NaCl, up to 4 times isotonic, has little effect on the Qo_2. Hypotonic solutions cause a definite decrease which is roughly progressive with the hypotonicity to 1/3 depression at 1/4th isotonic and 2/3 depression in water.

Attempts to maintain a constant CO_2 pressure of 40 mm. in the chamber, by a special absorber for CO_2 that would be effective at higher CO_2 tensions, were not satisfactory. The effect of Na HCO_3 added to NaCl was determined by comparison with NaCl solutions brought to the same pH (8.0-8.5) with borate or phosphate buffers. Certain theoretical difficulties with this procedure cannot be discussed here, but consistent results were obtained. The bicarbonate values fell between the other 2 sets, and no definite stimulating action can be ascribed to the HCO_3^- ion.

Glucose and glucose plus insulin added to a saline medium regularly had no influence on oxygen consumption; but in a few scattered experiments an increase of several hundred percent was ob-

[3] Fenn, *Medicine*, 1928, vii, 433.

[4] Feng and Gerard, PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii.

tained. Sodium lactate was also without influence in most cases but gave a 25% increase in one series of 3 experiments. The bases for these exceptional results have not been discovered. Addition of methylene blue markedly increases the Qo_2, 40% in one series on summer frogs, 70% in experiments the following spring. The methylene blue action is further enhanced by addition of sodium lactate, which caused a 95% increase (compared to 70%). The relation of methylene blue to lactic acid oxidation has been emphasized by Barron.[5] Although methylene blue penetrates the nerve sheath very poorly, experiments on nerves with split or intact sheaths gave identical figures. Cyanide, even in N/100 concentration, does not abolish more than 2/3 of the oxygen consumption, and methylene blue partly restores that lost.

Morphine (0.001 to 0.1%) has no influence on nerve respiration. Urethane causes 50% inhibition at a concentration of 5%, chloralhydrate at a concentration between 0.05 and 0.1%. The relative effectiveness of these narcotics in producing nerve block apparently cannot be correlated with their action on respiration (see also Sherif[6]). Bile salts, at a concentration of 125 mgm. %, slightly decrease the Qo_2 (Ries and Still[7]).

Bull frog sciatics have an average Qo_2 of 29 at 21°C. (Green frog nerves in comparative experiments averaged 45.) Rabbit sciatics at 38°C. average 280, but during the first hour higher values (320) are obtained and the Qo_2 slowly falls with time. Dog sciatics averaged 120 at 38° and 30 at 22°. Some of these had been allowed to degenerate a week with no distinct change in resting Qo_2. The dog nerve values may be low because the nerves were obtained from animals kept under barbital for some days.

The increased oxygen consumption by green frog sciatics on stimulation is being studied by Mr. T. H. Chang. The average of 40 experiments for continuous tetanization at 22°C. is 15. Splitting the sheath has no effect. At 280 shocks per second the increase is hardly 25% greater than at 100 per second. The increase on stimulation is abolished when only a small bit of nerve on the electrodes is stimulated, the remainder being cut off. This confirms my previous experiments in showing that the extra oxygen consumption depends on the activity of the conducting nerve and is not a local stimulus effect as suggested by Winterstein.[8, 9] Methylene blue does

[5] Barron, *J. Biol. Chem.*, 1929, lxxxi, 445.

[6] Sherif, *J. Pharm. and exp. Therap.*, 1930, xxxviii, 11.

[7] Ries and Still, *Am. J. Physiol.*, 1930, xci, 609.

[8] Winterstein, *Handb. d. norm. u. pathol. Physiologie*, 1929, ix, 393, Springer, Berlin.

[9] Holmes, Gerard and Solomon, *Am. J. Physiol.*, 1930, xciii, 342.

not affect the oxygen consumption of activity. This is in harmony with the established differences between the carbohydrate metabolism of nerve during rest and activity.

5111

The Standardization of the Hemolytic Index.

ARTHUR WEIL AND DANIEL M. LIPSCHUTZ. (Introduced by S. W. Ranson.)

From the Institute of Neurology, Northwestern University.

During our studies of the effect of hemolytic amboceptors on different tissues the necessity arose for the creation of a standard for the comparison of the hemolytic index of different seras. Everyone who has worked on similar problems has no doubt encountered the disadvantages of the present system of plus symbols. Not only is it subject to the personal interpretation of the investigator, but the varying degree of the hemolytic index of a given serum will depend on the age of the serum and the temperature of preservation. Furthermore the following variants of the blood cells have to be taken into consideration: Condition of the animal at the time of taking the blood, methods of preventing coagulation, differential count of the different blood-elements, volume of the red blood-cells, time of centrifugation, temperature during centrifugation, and, the most important factor of all, the increased sensitivity of the red blood cells, which rapidly increases with the time of preservation.

In order to be independent of all these variants it was thought desirable to have a standard hemolytic chemical agent, the action of which might be compared with the hemolytic effect of a given amboceptor. For this purpose sapotoxin was prepared from Quillaja bark saponin (Merck, crude), following with minor variations the methods described by Kobert and Brandl.[1] Sapotoxin has the advantage of being more effective than saponin and the methods of purification guarantee a more uniform product than the different commercial saponins.

Two systems were prepared. The first one consisted, as usual, of the hemolytic serum in varying amounts plus complement (guinea pig), plus 1 cc. of a 5% emulsion of red blood cells, which had been washed 3 times with a 0.85% NaCl solution. The second system consisted of varying amounts of sapotoxin plus 1 cc. of the same

[1] Brandl, J., *Arch. f. Exp. Path. u. Pharm.*, 1904, liv, 245.

5% emulsion of blood cells in each test tube. It had been found that a 0.05% solution of sapotoxin in a 0.85% solution of NaCl was best suited for this purpose. It was prepared in each experiment fresh from a standard 1% solution, to which 0.1% of salicylic acid had been added.

After both systems had been kept together for 2 hours in the incubator at 37°C., in both of them the test tube was determined in which complete hemolysis had occurred. It was assumed that the amount of serum which could produce complete hemolysis was equivalent to the amount of sapotoxin which had produced the same effect. For practical purposes that amount of sapotoxin was calculated in milligrams which was equivalent to the hemolytic action of 100 cc. of serum. This figure was called "Sapotoxin Units". Example: In the first system 0.025 cc. of serum produced complete hemolysis of 1 cc. of 5% blood. In the second system 0.290 cc. of the sapotoxin solution had the same effect. 0.290 cc. of a 0.05% solution of sapotoxin are equal to 0.145 mg. sapotoxin. The sapotoxin units are $0.145 \times 100/0.025 = 580$ sapotoxin units.

TABLE I.*

Sapotoxin units of different hemolytic amboceptors. Blood was injected intra-abdominally in intervals of three days. The serum was taken nine days after the last injection.

Blood from	Injected into	Amount of blood injected		Sapotoxin Units
		%	cc.	
Rat	Dog	50	3-3-3-3	95
Rat	Dog	50	4-8-12	315
Cow	Dog	50	10-10-10	800
Cow	Dog	50	10-10-10-10	1070

* E. Sieburg. *In Handbuch der biologischen Arbeitsmethoden*, E. Abderhalden Abt. I, Teil 10, pp. 545-584. 1923.

5112

Denaturation of Hemoglobin.

ARTHUR G. COLE AND ALDEN K. BOOR. (Introduced by W. H. Welker.)

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In a recent series of papers by Anson and Mirsky,[1] the claim is made that hemoglobin, in N/20 HCl, is completely denatured in

[1] Anson, M. L., and Mirsky, A. E., *J. Gen. Physiol.*, 1930, xiii, 121, 133, 469, 477.

3 minutes at 0°C. These authors claim, further, that this denaturation may be reversed to the extent of 75% by simply neutralizing the acid added with an equivalent amount of NaOH containing a small amount of NaCN. These experiments on the reversal of hemoglobin denaturation are, in essential details, substantially the same as those published almost 3 years earlier by Wu and Lin.[2] Wu and Lin, however, considered that they were dealing not with true denaturation but with a pseudo-denaturation which was peculiar to hemoglobin and dependent upon its prosthetic group. In order to test these divergent views, we repeated the experiments of Anson and Mirsky at temperatures which would really denature the protein. Instead of insolubility at the iso-electric point, we made use of precipitin reactions against a specific antiserum to follow the course of denaturation. Since true denaturation of a protein results in a gradual diminution of its ability to react with its antiserum, we may expect that any reversal of this denaturation would manifest itself by a return of its precipitinogenic properties.

A 3% solution of ox carbon monoxide hemoglobin, prepared by the method previously described by Boor and Hektoen[3] was used in these experiments. One volume of this solution was treated with 3 volumes of N/15 HCl and, after standing at room temperature for 10 minutes, the brown mixture was placed in a boiling water bath. At stated intervals, 4 cc. of this mixture was removed and neutralized by the addition of 1 cc. N/5 NaOH and 1/20 cc. N. NaCN. After standing for one hour at room temperature, 3 cc. of this solution was treated with an equal volume of saturated ammonium sulphate solution while the remainder was used for precipitin reactions against an ox carbon monoxide hemoglobin antiserum. Similar portions of solution, taken at the same time, were also permitted to stand at room temperature for one hour and then used, without neutralization, for precipitin and ammonium sulphate precipitation reactions. The results obtained in a typical series of experiments are summarized in the table.

These preliminary experiments indicate very definitely that heating of the acid solution results in a gradual loss of ability to react with the specific antiserum, in agreement with results observed in the denaturation of other proteins. Parallel experiments heated the same length of time, one neutralized while the other was not, show practically no differences in precipitin titer in spite of the fact that the unneutralized solutions had stood for an additional hour at room

[2] Wu, H., and Lin, K., *Chinese J. Physiol.*, 1927, i, 219.

[3] Boor, A. K., and Hektoen, L., *J. Infect. Dis.*, 1930, xlvi, 1.

TABLE I.

Denaturation of Ox Carbon Monoxide Hemoglobin.

3% solution of ox carbon monoxide hemoglobin treated with 3 volumes of N/15 HCl. Solution placed in boiling water bath after 10 minutes at room temperature.

Exp. No.	Time of Heating in min.	Solution neutralized with	Precipitin Reactions with anti-ox COHb serum	Half Saturation with $(NH_4)_2SO_4$	
				Ppt.	Filtrate
1	5	NaOH+NaCN	+++[1]	Slight	Red
2	5	——	+++	Heavy	Colorless
3	15	NaOH+NaCN	++	Small	Light Red
4	15	——	++	Heavy	Colorless
5	30	NaOH+NaCN	++	,,	Pale Red
6	30	——	++	,,	Colorless
7	60	NaOH+NaCN	+[2]	,,	Faint Red
8	60	——	+	,,	Colorless
9	135	NaOH+NaCN	+	,,	,,
10	135	——	+	,,	,,
	Room Temp.				
11	10	NaOH+NaCN	+++	Very slight	Deep Red
12	10	——	+++	Heavy	Colorless
13	60	——	+++	,,	,,
14	Control with ox COHb		+++++[3]	No ppt.	Deep Red

[1] Highest dilutions of test solutions which gave precipitates with antiserum in 1 hour at room temperature. + = dilution of 1 to 10, ++ = dilution of 1 to 100, +++ = dilution of 1 to 1,000, etc.

[2] Probably represents a non-specific reaction since most solutions also gave precipitates with normal rabbit serum at this dilution.

[3] Control gives precipitate with antiserum in dilution of 1:13,300,000.

temperature before the precipitin reactions were carried out. The precipitation reactions with ammonium sulphate, which Anson and Mirsky use as an index of the extent of reversal of denaturation, show that the amount of hemoglobin, as indicated by the color in the supernatant liquid, decreases with time of heating until, in about one hour, the supernatant liquid is colorless. These results differ markedly from those obtained in similar experiments at room temperature. In contrast with the neutralized solutions, all of the protein in the acid solutions is completely precipitated by half saturation with ammonium sulphate. That this result is peculiar to hemoglobin is indicated by the fact that hemoglobin, treated with HCl as above at room temperature and then immediately half saturated with ammonium sulphate is also completely precipitated, whereas crystallized ovalbumin gives practically no precipitate under similar conditions.

We are thus led to the conclusion that Anson and Mirsky, in their experiments, dealt not with true denaturation but with an even more obscure reaction which is peculiar to hemoglobin. We find no evidence for reversal of denaturation, by their method, when the pro-

tein is subjected to conditions which actually denature it. Although Anson and Mirsky consider hemoglobin a typical coagulable protein, they do not differentiate between coagulation and denaturation. We cannot agree with their point of view that the effects of acid on hemoglobin are the same as on other coagulable proteins. Besides a conceivable denaturation of the entire hemoglobin molecule, acids exert other effects such as the splitting of hemoglobin into hematin and globin and the denaturation of the globin which are characteristic of hemoglobin and are entirely absent when dealing with the simple proteins. We feel that insolubility at the iso-electric point cannot be used as a criterion for denaturation of hemoglobin because of the complex transformations which this particular protein undergoes upon treatment with acids. Such a physical property, though valuable in the case of simple proteins like ovalbumin, is of little significance in the case of hemoglobin because of a lack of definite information on solubility and other relationships between the various components of the reaction mixtures.

5113

Some Observations on the Growth of Rats on "Fat-Free" and Fat-Containing Diets.

R. G. SINCLAIR. (Introduced by W. R. Bloor.)

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Recently Burr and Burr[1] and McAmis, Anderson, and Mendel[2] have shown that the growth of rats on a diet from which all true fat has been excluded is distinctly inferior to that of rats on a diet which contains some fat, even though the fat used is devoid of vitamin A. Furthermore the health of the animals raised on the fat-free diet also suffers. Burr and Burr[1] believe that the total deprivation of food fat leads to the development of a deficiency disease, the symptoms of which are impaired growth, scaliness of the feet and tail, excessive production of dandruff, with ultimate hematuria and albuminuria, loss in weight, and premature death. In a later paper[3] Burr and Burr state that fat is an essential constituent of the diet

[1] Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1929, lxxxii, 345.

[2] McAmis, A. J., Anderson, W. E., and Mendel, L. B., *J. Biol. Chem.*, 1929, lxxxii, 247.

[3] Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1930, lxxxvi, 571.

because of the inability of rats to synthesize sufficient quantities of the more highly unsaturated fatty acids, specifically linoleic acid, for the maintenance of the normal functioning of the tissue lipids.

In the present paper the author wishes to present a few observations which would seem to have a significant bearing on the question of the rôle of fat in animal nutrition, especially with respect to the theory that linoleic acid and other highly unsaturated fatty acids are essential constituents of the diet. For the most part these observations are purely incidental, having been made on rats raised on various diets in connection with a study of phospholipid metabolism.

In the earlier phase of the work, all rats on experimental diets were raised individually in cages constructed throughout of No. 2 mesh wire screen and raised on 2-inch legs. Without exception, the rats which were being fed on the "fat-free" diet* developed a peculiar scaly condition of the tail similar in every respect to that described and illustrated by Burr and Burr.

However, since the need for animals raised on the "fat-free" diet exceeded the supply of false-bottom cages, it was decided to raise groups of rats in the ordinary stock cages. This necessitated a change in the method of feeding. Heretofore the extracted yeast and the Oscodal† had been fed separately from the main ration but it seemed advisable to mix the vitamin supplements and the basic ration if the animals were to be raised in groups. Accordingly the "fat-free" diet which has been fed to animals in the stock cages contains 2.5 gm. of yeast and 0.025 gm. of Oscodal for each 100 calories in the basic ration.

In view of the unfailing occurrence of scaliness on the tails and feet of rats fed essentially the same diet in the false-bottom cages, it was quite unexpected to find that the rats in the stock cages remained quite normal in appearance. To date between 45 and 50 rats have been raised on the "fat-free" diet in the stock cages and in not a single instance has there been any sign of scaliness or of any other abnormality in appearance. However, the rate of growth has been noticeably slower than that of rats on the stock diet of kitchen scraps. Most of the animals were killed when they reached a body weight of about 150 gm. so that it is impossible to say whether or not these animals would have continued in apparent good health

* The basic "fat-free" diet is identical in composition to No. 550 of Burr and Burr.[1] The casein is extracted in a continuous extractor with boiling alcohol, the yeast with ether in a Soxhlet apparatus. By analysis of the ingredients the mixed "fat-free" diet contains about 0.25% of fatty acids.

† The nonsaponifiable matter of cod liver oil, kindly supplied by the H. A. Metz Laboratories, Inc., through the courtesy of Dr. H. E. Dubin.

throughout the normal life cycle. However, one group of rats, now 4 months old, is still quite normal in appearance, although the average weight is sub-normal.

There seemed to be only two factors which could be responsible for the absence of scaliness from the rats raised on the "fat-free" diet in stock cages: one, the mixing of the yeast and the Oscodal with the basic ration; and the other, the fact that in the stock cages the animals were bedded with paper and had access to their feces. In order to see which explanation was correct the following experiment was begun: Nine littermate rats were divided into two groups. One group was placed in the false-bottom cages constructed throughout of No. 2 mesh wire screen; the other group was placed in similar cages over the bottoms of which window screening (No. 16 mesh) had been placed in order to retain the feces. Of each group, 3 rats were placed together in a cage, the others being kept in individual cages. All were fed on the same "fat-free" diet in which the yeast and Oscodal were mixed with the basic ration. These rats are now 23 weeks old and have been on the diet for 19 weeks. All of the rats in the cages with the No. 2 mesh wire bottoms have developed well marked scaliness of the tail and, in some instances, node-like constrictions which eventually caused a small piece of the tail to blacken and drop off. On the other hand, none of the 4 rats which have had access to their feces has developed any scaliness of the tail.

Now, although there is an unmistakable difference in the appearance of these two groups of rats, there has been no essential difference in their growth, all being definitely subnormal in weight. The poor growth of this particular group of rats is not entirely due to the absence of fat from the diet since rats fed on the same diet but raised in stock cages have grown very much better.

The question is whether or not the substance which is present in the feces (possibly in the bacteria) of animals on a "fat-free" diet is a highly unsaturated fatty acid, the consumption of which is responsible for the absence of scaliness from the rats raised in the stock cages. While the author has not been concerned with an intimate investigation of the phenomenon, certain incidental evidence has been obtained which is difficult to harmonize with such an explanation.

It has already been shown[4] that the constituent fatty acids of the phospholipids in the tissues of rats raised on a "fat-free" diet have a low degree of unsaturation as compared with those of stock rats or of rats fed on a diet containing olive oil. Recent work has con-

[4] Sinclair, R. G., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 793.

firmed this fact. Furthermore it has been found (unpublished experiments) that small amounts of cod liver oil added to the "fat-free" diet (60 mg. daily or 1% by weight) increase the iodine numbers of the phospholipid fatty acids from the level of 100, characteristic of the "fat-free" diet, to about 125, while 1% by weight of lard gives an I.N. of 115. With this fact in mind, one should expect to find a higher I.N. in the phospholipid fatty acids in rats raised in stock cages and therefore normal in appearance, than in rats raised in false-bottom cages and showing marked scaliness of the tail, if the protective action of the feces is due to a highly unsaturated fatty acid. Values for the I.N. of the phospholipid fatty acids of rats raised on the "fat-free" diet are as follows: rats showing marked scaliness 99, 99, 104; rats quite normal in appearance 100, 104, 100, 105. Since there is no difference it seems hardly likely that the rats raised in the stock cages have consumed with their feces appreciable quantities of unsaturated fatty acids.

Furthermore it has been found that rats raised in false-bottom cages and fed on the "fat-free" diet to which cod liver oil has been added (even to the extent of 10% by weight) develop marked scaliness of the tail. The scaliness in this case is certainly not due to a low degree of unsaturation in the tissue lipids since the latter are quite highly unsaturated. Lard, on the other hand, seems to be effective in preventing the scaliness when present to the extent of 1% of the diet.

While the evidence available at present seems to be rather against the probability that the failure of rats fed on a "fat-free" diet to develop scaliness if they are raised in stock cages is due to the consumption with the feces of appreciable quantities of a highly unsaturated fatty acid, the final solution of the problem will come only from direct experimentation. Since the author does not intend to undertake further investigation of this problem, it has seemed advisable to place the above observations on record.

5114

Concentration and Purification of Antityphoid Horse Serum.

MIRIAM REINER AND GREGORY SHWARTZMAN.

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Extremely severe hemorrhagic necrosis appears in rabbits at the skin sites prepared by an injection of a bacterial filtrate when the injection is followed 24 hours later by an intravenous injection (*i. e.,* reacting factors) of a culture filtrate of the same or another bacterium.[1, 2, 3, 4] Among the various phases of this phenomenon of local skin reactivity, the specific neutralization of the reacting factors by homologous immune sera was observed. A method has been described for quantitative measurement of the neutralizing antibodies of immune antimeningococcus and antityphoid horse sera. Inasmuch as the titer of these antibodies did not run parallel to the agglutination titer of the same sera, advantage was taken of this method[2, 3] in order to develop highly potent neutralizing horse sera for ultimate therapeutic application. The work reported below was undertaken in order to concentrate and purify the antityphoid neutralizing antibodies.

Concentrated and purified antibody solutions should contain a maximum amount of antibodies associated with a minimum of total solids, especially of protein, as indicated by the nitrogen content. Gibson and Banzhaf[5, 6] were able to obtain three- to fourfold concentration of diphtheria and of tetanus antitoxins from antisera. Preparations of pneumococcus antibody solutions by methods of Banzhaf,[7] and Banzhaf and Sobotka,[8] and by other methods, yield concentrations up to 7-10 times the amount of protective units found in the original serum. It should be noted that only solutions containing the identical amount of total solids or nitrogen may be directly com-

[1] Shwartzman, G., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 560; xxvi, 131, 207; *J. Exp. Med.*, 1928, xlviii, 247; 1929, xlix, 593; 1930, li, 571; *J. Inf. Dis.*, 1929, xlv, 232; *Klin. Wsch.*, 1930, *Ein neues immunologisches Phaenomen*, in press.

[2] Shwartzman, G., *J. Exp. Med.*, 1929, i, 513; *J. Am. Med. Assn.*, 1929, xciii, 1965.

[3] Shwartzman, G., "The effect of bacterial variations upon the factors necessary for the phenomenon of local skin reactivity" (in preparation).

[4] Shwartzman, G., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 843.

[5] Gibson, R. B., *J. Biol. Chem.*, 1906, i, 161.

[6] Gibson, R. B., and Banzhaf, E. J., *J. Exp. Med.*, 1910, xii, 411.

[7] Banzhaf, E. J., *Weekly Bull. N. Y. Dept. of Health*, 1927, xvi, 17.

[8] Personal communication.

pared. By the following method we are able to reach a much greater concentration as demonstrated by the phenomenon of local skin reactivity to *B. typhosus*.

The plasma or serum was diluted to one and a half times its original volume with distilled water. Instead of a saturated ammonium sulfate solution, a 47.5% sodium-magnesium sulfate solution was used as the protein precipitant to facilitate the nitrogen analysis. Twenty-eight parts of this solution were added to 72 parts of the diluted serum. The precipitate which contains fibrinogen and part of the euglobulin was discarded. Then a sufficient amount of the salt solution was added to the filtrate to bring the sulfate concentration up to 19%. The precipitate obtained was allowed to become almost dry on the filter paper and was then transferred to a dialyzing bag in running water. After about 6 hours the white pasty mass became a clear brown solution which gradually grew turbid and later showed a white precipitate. The dialysis was continued for 72 to 96 hours until all the sulfate ion had disappeared and no more precipitate seemed to form. The globulin solution was centrifuged and the precipitate was made up with physiological salt solution to a convenient volume, *e. g.*, one-tenth of the original serum. Extraneous matter was removed by centrifuging, and the supernatant fluid filtered through a Berkefeld "V" candle.

The neutralization experiments were performed as follows: (a) Titration of *B. typhosus* reacting factors. *B. typhosus* culture filtrates employed in this work were prepared according to the method previously described.[4] The rabbits used for titrations were each injected intradermally with 0.25 cc. of the undiluted filtrate* and divided into groups of 4. Twenty-four hours later a single intravenous injection of the filtrate diluted in 0.85% NaCl solution (one cc. per kilo of body weight) was given to each rabbit. Each group received intravenously a different dilution of the filtrate. The local reactions were read 4 to 5 hours after the intravenous injection. The titrations were continued until the lowest dilution was found which gave no reactions in the 4 rabbits tested. The highest dilution which gave reactions in one or more rabbits of the group was also ascertained. The minimal dose of reacting factors was then considered to be between these 2 figures.

(b) Measurement of neutralizing antibodies. The technique employed for the neutralization experiments was similar to one described before.[2, 3] One area of the skin of the abdominal wall of

* The standardization of this procedure will be discussed in detail in a subsequent publication.

the rabbit was injected with 0.25 cc. of the undiluted filtrate. Twenty-two to 24 hours later a single intravenous injection was made of a mixture of the same filtrate (diluted previously in 0.85% NaCl solution to the desired degree) with a given undiluted serum in the proportion 4:1. The mixtures prepared on the morning of the experiment were incubated in a water bath at 37.5°C. for one hour. The precipitate in the mixtures was broken up by shaking immediately before the injection. The intravenous dose was 1.25 cc. per kilo of body weight. Each mixture was tested in 4 rabbits. The

TABLE I.
Concentration and Purification of Anti-typhoid Horse Sera.

Sample	Vol. in cc.	N mg. per cc.	Agglutinins	Precipitins	Neutral units per cc.	Neutral units per mg. nitrogen	Total No. neutralizing units	Yield of neutralizing antibodies
Original horse serum No. 110A	200	11.3	10240	32	580	50	116000	52%
Antibody solution recovered	75	0.3	6400	16	800	2650	60000	
Concentration per mg. N			23X	19X		53X		
Original horse serum No. 110A	480	11.4	10240	32	580	50	278400	40%
Antibody solution recovered	96	0.3	20480	64	1200	4000	115200	
Concentration per mg. N			76X	76X		80X		
Original horse serum No. 133	450	11.5	10240	32	580	50	261000	45%
Antibody solution recovered	45	1.1	102400	128	2600	2350	117000	
Concentration per mg. N			105X	42X		47X		
Original horse serum No. 133	350	11.5	12800	16	360	30	126000	44%
Antibody solution recovered	35	1.3	25600	64	1600	1200	56000	
Concentration per mg. N			18X	36X		40X		
Original horse serum No. 133	350	11.5	12800	16	360	30	126000	44%
Antibody solution recovered	35	1.9	25600	64	1600	850	56000	
Concentration per mg. N			12X	24X		28X		

amount of serum (0.25 cc. per kilo of body weight) was kept constant. The titrations were continued until the lowest dilution of the filtrate was found which was still consistently neutralized (*i. e.*, in all rabbits tested) by this amount of serum. The number of reacting units present in this lowest dilution of the filtrate was taken to indicate the same number of neutralizing units in 0.25 cc. of the serum.

The volume, nitrogen content, number of neutralizing units, agglutinins and precipitins of 2 batches of antityphoid horse sera and of 5 representative concentrated preparations are compared. The total yield in neutralizing antibodies was from 40 to 50% of that in the original serum. The concentration as indicated by the quotient Neutralizing Units/mgm. N varied from 28 to 80 times. Thus a great part of the neutralizing antibodies was recovered in a very small globulin fraction. The range of magnitude of this concentration exceeds by far any concentration attained by previous authors by chemical separation of antibodies of various antibody and antitoxin solutions. There is apparently no relation between the concentration of the agglutinins and the neutralizing antibodies. Further observations will decide whether a parallelism exists between neutralizing antibodies and precipitins.

Further work on antityphoid as well as on antimeningococcus and anticoli horse sera is under progress.

Preliminary chemical analysis of this highly specific serum fraction seems to indicate the preponderance of proteins in its constitution. Dr. Harry Sobotka and one of the authors will report this phase of the work in another communication.

5115

Chorio-allantoic Grafting Followed by Direct Transplantation in the Chick.*

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The present note calls attention to the possibilities of a new procedure in tissue grafting which promises to prove useful in investigation of problems in development and which should be made avail-

* From the Department of Anatomy, Harvard Medical School; to which the author is indebted for the material and facilities.

able to other workers in this field. The technique combines the method of grafting into the chorio-allantoic membrane as done by Willier[1] and others with that of skin transplantation as described by Danforth and Foster.[2]

The writer is indebted to Professor P. D. F. Murray of Sidney for the information[3] that in his numerous experiments in chorio-allantoic grafting he has at times included pieces of skin which occasionally produced down differing in color from that of the host. As suggested by Professor Murray such experiments are of much value in the study of potentialities in down production during the period of incubation. But they are limited by the fact that each automatically terminates with the hatching of the host and loss of its allantois.

With the hope of finding a way to overcome this handicap the following experiment was undertaken. Twenty-four White Leghorn eggs were put in the incubator at 12 M. on May 7. On May 9, at 3 P. M., 12 Barred Plymouth Rock eggs were put in the same incubator. On May 16, between 3 and 6 P. M., all of the Plymouth Rock eggs were opened but found to contain only 2 living embryos, one of which showed ectopic viscera and undoubtedly would have died in a day or two. These embryos were taken out in warm salt solution and grafts prepared from each. The transplants were made in the usual manner into the membranes of 10 Leghorn embryos. One control and 2 operated eggs hatched on May 27. In one of the latter the graft to the allantois had apparently failed, but attached to the shell of the other, at some distance from the site of operation, was a small mass which on dissection proved to consist of bone, connective tissue, muscle(?) and a considerable amount of skin, on which there were about 40 dark-colored down feathers. This skin was carefully dissected free in 2 small pieces, one of which was transplanted to the back of the original host, the other to the leg (tibio-fibular region), since this was the part from which the original transplant had come. (It represented about half of one leg of the defective embryo.)

The grafted chick made a good start toward normal development but was accidentally killed on the sixth day following the second operation. The skin was immediately removed and examined under low power magnification, when it was found that the graft to the back had failed and the one on the leg had pulled in two, the prox-

[1] Willier, B. H., PROC. SOC. EXP. BIOL. AND MED., 1925, xxxiii, 26.

[2] Danforth, C. H., and Foster, Frances, PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, 27.

[3] In a personal communication.

imal part of this also having failed. But the distal part, which bore several typical Plymouth Rock down feathers, was thoroughly healed in and apparently entirely normal. In the light of previous results from skin grafting there seems no reason to suppose that this part of the graft would not have persisted as long as the host might live.

This lone experiment is of interest (a) in showing incidentally that tissues of a defective embryo may, under favorable conditions, far outlive the life expectancy of the embryo itself; (b) in confirming the observation that at the time the first down follicles are forming, or even before (at the age of 7 days, 3 hours in this case), the skin appears to have already acquired some of its definitive potentialities; and, especially, (c) in indicating the possibility of bridging the gap between the periods before and after hatching in such a way as to make it seem feasible to study the behavior of feather follicles in foreign environments from the time they are first formed in embryonic skin through to adult life.

5116

A Method for the Exclusion of Liver Function.

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When the patency of the common bile duct is destroyed, as by a stricture, or by the pressure of an inoperable tumor of the head of the pancreas, the surgeon is confronted with an operation of necessity; an exit must be provided for the bile. Various procedures have been suggested, and are applicable according to the actual conditions found in the given case. The most usual procedure is the operation of cholecystenterostomy—a direct anastomosis between the gall bladder and some portion of the digestive tube.

This is a life saving procedure, easily carried out, with quite satisfactory results. Perhaps the only objection is that the pressure within the digestive tube is greater than the pressure in the gall bladder, with the consequence that the intestinal content is forced into the biliary system, with sometimes a final result of dilatation and general cholangitis.

This untoward final result of the accepted operative procedure led to the following: The intestine is sectioned at some suitable

TABLE I.

Blood	Normal	Duct Tied	Duct not tied
Cholesterol	160	278.3	175.5
Urea N_2	12	20.7	12.0
Chlorides	590	438.0	538.2
Sugar	99	80.1	100.0
Calcium	9	14.0	10.0
Uric Acid	trace or 0	2.8	0.0
Icteric Index	2-3	12.0	2-3
Van den Bergh	neg.	direct, prompt	neg.
Bile Salts	normal	50-100% increase	normal
Spectroscopic exam. of blood serum	Slight bile pigment	Increased bile pigment	Slight bile pigment
Urine			
Bile pigment	0	++ 2nd day	0
Urobilinogen	0	0	0
Amino acids	Trace	Increased	0
Spectroscopic	Neg.	Bile pigment	0

point, and the distal end united to the gall bladder by an end-to-end anastomosis (A) with the fundus of the gall bladder. The continuity of the digestive tube is now restored by a suitable end-to-side anastomosis of the proximal end of the tube to the side of the intestine at a point 7 or 8 inches below the end-to-end union between gall bladder and distal intestine (B). The purpose of this procedure is to interpose between the gall bladder and the stream of intestinal content a segment of intestine in which the direction of normal peristaltic activity would act as a one-way valve, tending to prevent the entry of intestinal content into the gall bladder.

The operation is not difficult, offering, neither in complication of procedure nor in time required, any explanation of the curious result. In a large series of experiments, all the animals have died within 48 hours, with the symptoms of an acute liver insufficiency, provided the common duct is doubly ligated, and cut between the ligations—as would be done naturally in an experiment designed to duplicate the clinical conditions for which the procedure is suggested. If the common duct is not ligated, the animal does not die.

It is not proposed to discuss the possible reasons for the results. The experiments of Werelius[1] are of importance. This report is presented since the method seems to offer a means of excluding certain liver functions, without the profound disturbance of other functions connected with the liver, such as glycolysis, which appears when the liver is extirpated.

The effect upon the blood chemistry is best shown in the appended table.

[1] Werelius, A., *J. Am. Med. Assn.*, 1916, lxxix, 535.

5117

Seasonal Variation in Efficiency of New Orleans Sunshine and Skyshine in Preventing and Curing Rickets in Rats.*

H. S. MAYERSON AND HENRY LAURENS.

From the Laboratory of Physiology, School of Medicine, Tulane University of Louisiana, New Orleans.

Rats, fed the Steenbock and Black rickets-producing diet, were divided into groups at the time of weaning and exposed to sunshine and skyshine for varying lengths of time between 10:00 A. M. and 1:00 P. M. Diagnosis was based on line tests, roentgenograms, and blood phosphorus determinations. The duration of the experiments was usually 5 weeks, being prolonged when the controls did not show florid rickets within this time.

During June, 1929, an average daily exposure to sunshine of 3 minutes prevented rickets, during July 9.5 minutes and during October 5 minutes. The average daily exposure to sunshine is frequently less than the actual time out of doors because of cloudy weather. Since no indications of rickets were present shorter exposures would certainly have been sufficient. From November 16 to December 20, 1929, an average daily exposure of 10 minutes afforded protection but 5 minutes did not. From December 5 to January 17 rats given an average daily exposure of 14 minutes developed mild rickets, as did those exposed for from 2 to 5 minutes from January 17 to March 31. An average daily exposure of 6 minutes from March 29 to May 1, however, was protective.

During June, 1929, an average exposure to skyshine for 8 minutes daily was insufficient. During July, 24 minutes was borderline, and 48 minutes afforded complete protection. During October, November and December exposures of 22 to 24 minutes were protective, as was an average daily exposure of 67 minutes from January 3 to February 14. From February 10 to March 24 incomplete protection was afforded by 28 minutes' exposure, while 47 minutes during April was just borderline.

Such observations will be made for several years. Rats are also being exposed under some of the ultraviolet transmitting screens. In these observations and in those on chicks the total amount of energy and its spectral distribution was determined nearly every time the animals were exposed.

* Aided by a grant from the David Trautman Schwartz Research Fund of Tulane University.

5118

Seasonal Variation in Efficiency of New Orleans Sunshine and Skyshine in Promoting Growth and Preventing Leg Weakness in Chicks.*

HENRY LAURENS AND H. S. MAYERSON.

From the Laboratory of Physiology, School of Medicine, Tulane University of Louisiana, New Orleans.

Groups of 2-week-old chicks, fed a leg weakness-producing diet, were exposed daily for 6 weeks to different amounts of sunshine and skyshine between 10:00 A. M. and 1:00 P. M. Growth was followed by weekly weighings. As judged by appearance, attitude, roentgenograms and blood calcium and phosphorus, an average daily exposure to sunshine of 4 to 5 minutes from October, 1929, through March, 1930, and of 2 to 3 minutes through April, 1930, prevented leg weakness. An average daily exposure to skyshine of 62 minutes protected the chicks from October, 1929, to the middle of February, 1930, 28 minutes sufficed from that time through March, 1930, and 23 minutes during April, 1930. We are confident that smaller amounts of radiation would have been effective. Better growth was obtained in the skyshine than in the sunshine animals from October 16 to January 10, indicating that the exposures to direct sunshine were, perhaps, too long.

5119

Classification of the Anemias on the Basis of Differences in the Size and Hemoglobin Content of the Red Corpuscles.

M. M. WINTROBE. (Introduced by J. H. Musser.)

From the School of Medicine, Tulane University of Louisiana, New Orleans.

From determinations of the number of red corpuscles, the quantity of hemoglobin, and the relative volume of packed red cells in any sample of blood it is possible to calculate the mean volume and the hemoglobin content of the red corpuscles of the sample.[1] The constants derived by these calculations are spoken of as mean corpuscular volume, mean corpuscular hemoglobin and mean corpus-

* This work was made possible through the generosity of Mr. J. S. D'Antoni.

1 Wintrobe, M. M., *Am. J. Med. Sci.*, 1929, clxxvii, 513; *J. Lab. and Clin. Med.*, (to be published).

cular concentration. Mean corpuscular volume is expressed in cubic microns. Mean corpuscular hemoglobin refers to the actual amount of hemoglobin contained in the average red corpuscle and is expressed in micromicrograms. Mean corpuscular concentration refers to the hemoglobin contained in the red corpuscle in proportion to its volume. In the determination of this constant the red corpuscle is presumed to contain an aqueous solution of hemoglobin the concentration of which is calculated and expressed in per cent.

Some 400 accurate blood determinations have been carried out in 140 patients suffering from anemia produced by a large variety of causes. On the basis of differences in the size and hemoglobin content of the red corpuscles observed in these cases, 4 distinct classes have been differentiated:

I. The first and most obvious group, which can be called *macrocytic,* includes the cases of pernicious anemia, sprue, and a case of pernicious anemia of pregnancy. The noteworthy characteristic of this group is an increase in the mean size and hemoglobin content of the red corpuscles. The increases in corpuscular volume and in corpuscular hemoglobin are proportional and thus the normal hemoglobin concentration of the cells is not disturbed. The increases in size and hemoglobin content vary more or less inversely with the red cell count but this correlation is not entirely strict.[2]

II. The second class, which may be called *"normocytic,"* is distinguished by a reduction in the red cell count without any change or at most only a slight increase in the size and hemoglobin content of the red corpuscles. Corpuscular concentration in this group is likewise normal. This class includes cases of anemia resulting from acute blood loss, malarial infection, and several cases of anemia of the aplastic or semi-aplastic type.

III. The third class, which I have termed the *"simple microcytic"* type of anemia includes the most commonly observed forms of anemia. When the reduction in the number of red corpuscles is only slight, no alterations in the size or hemoglobin content of the cells are observed in this class. When the anemia is more marked there is a slight reduction in mean corpuscular volume and in mean corpuscular hemoglobin. When the anemia is severe, the reduction in volume and corpuscular hemoglobin becomes more marked but at no time is this decrease as great as is the reduction in the number of red corpuscles. No matter what the degree of anemia in this class the corpuscular concentration remains normal or only very slightly reduced. The anemia associated with chronic infections and intoxica-

[2] Wintrobe, M. M., (to be published).

tions, such as bronchiectasis, pulmonary abscess, unresolved pneumonia, typhoid fever, cardiovascular renal disease, and carcinoma, when no blood loss was associated, was found to be of this type.

IV. The fourth class is distinguished by a marked reduction in the volume of the red corpuscles and a decrease in their hemoglobin content which is even more marked than the decrease in size. Even when the number of red corpuscles is not lowered a distinct reduction in their volume has been found in this group of anemias. The reduction in size in this class is even more marked than that observed in class III. The lowest values for mean corpuscular volume have been found in this group.

Even more characteristic than the reduction in the size of the cells in this type of anemia is the alteration in their hemoglobin content. The reduction in corpuscular hemoglobin is even more marked than the diminution in volume with the result that in this group, and apparently in this group alone, corpuscular concentration values significantly and constantly lower than normal are found. For this reason I have called this type of microcytic anemia *"hypochromic."*

The anemias which fall into this hypochronic, microcytic group are largely composed of those resulting from chronic blood loss. Since the cause of the anemia resulting from hookworm infestation is still disputed, it is of especial interest that the anemia observed in hookworm disease has been of this hypochromic type.

Further observations are being made with a view to determining the accuracy and usefulness of this classification, the relationship of the 4 classes of anemia to one another, and the effects of various forms of therapy on the size and hemoglobin content of the red corpuscles.

5120

Mechanism of Nerve Asphyxiation: With a Note on the Nerve Sheath as a Diffusion Barrier.

T. P. FENG AND R. W. GERARD.

From the Department of Physiology, the University of Chicago.

In the absence of oxygen nerve conduction is suspended in several hours, the activity of each fibre becoming gradually less until it blocks. Conduction has been shown to depend on oxidations, and the long persistence of activity with outside oxygen excluded to depend on the existence in nerve of an oxidizing reserve. The rapid recov-

ery from anoxia when oxygen is again admitted suggested that the nerve might similarly recover when some other reducible substance, such as methylene blue, were made available.

To study this, a double chamber was constructed to accommodate the 2 sciatics of one frog. Each nerve was stimulated at one end and the action potentials led from the other crushed end and the side. Between stimulating and lead electrodes the nerve traversed a sealed compartment, through which nitrogen could be passed, and opening through its floor to a bulb containing fluid. During the asphyxiation nitrogen entered the bulb from the bottom and, after bubbling through the liquid, swept through the nerve chamber. This rendered the liquid oxygen-free, while the nerve asphyxiated, and compression of the bulb then bathed the nerve with this liquid. One side contained Ringer solution buffered at pH 7 or 8, the other a similar solution plus 0.5% methylene blue or 1-naphthol 2-sulphonate indophenol. It was regularly found that neither solution brought about recovery. (The action potentials are led from nerve in air below the exposed region and are an index, therefore, of the number of fibre-impulses getting through, not of the impulse intensity in the experimental stretch.)

We then observed that methylene blue, though staining the nerve sheath deeply, hardly penetrated to the bundle of axones. It is not difficult to split the sheath longitudinally with little injury to the conducting elements, and in subsequent experiments this was done. The asphyxiated nerve then showed a definite recovery in methylene blue and also in Ringer solution, the action potentials returning to about 1/3 their original value. (This does not mean that each fibre recovered from zero activity to 1/3 normal.)

The great importance of the perineurium as a diffusion barrier is seen when the time to block by various agents (KCl, $CaCl_2$, NaCN, glucose) is determined for the nerves with intact and split sheaths (Table I). It may be noted that the agents producing most rapid block are least rapidly recovered from in Ringer.

TABLE I.

Nerve in	Intact Nerve		Sheath Split	
	Block	Maximum recovery in Ringer	Block	Maximum recovery in Ringer
	min.	min.	min.	min.
Isotonic glucose	None in 3 hrs.	<1	17	<1
Isotonic $CaCl_2$	50+	20	<2	9
Isotonic KCl	15	50	<2	20
N/150 NaCN in Ringer	80	—	10	—

Since even Ringer led to some recovery from asphyxial block a new method was used to determine the methylene blue effect. The stretch of nerve was asphyxiated in Ringer solution by bubbling nitrogen through and then solid methylene blue, lying on a plate at the top of the compartment, was tipped in. No recovery was obtained with this dye or the indophenol. A final series of experiments, with oxidations of a stretch of nerve interfered with by cyanide, also yielded no difference when methylene blue was present.

The negative results with methylene blue cannot be due to lack of penetration—the axones were well stained, nor too low an oxidizing potential—the nerve does reduce methylene blue and the more powerful indophenol is not more effective. Probably the energy available is not sufficient or is liberated largely at a stage in the chain of reactions or a region of the cytoplasm where it cannot be utilized.

Asphyxial block of nerve must represent a suspension or diminution of energy yielding reactions (oxidations). This might be the result of (1) exhaustion of reactants (oxidizing reserve depleted), (2) accumulation of end products, or, (3) alteration of the mileau so that the conditions permitting these reactions at a necessary rate no longer obtain. Of course all factors may contribute to lowering below a critical value for conduction. When a nerve first blocks in nitrogen it cannot be entirely due to (1) or bathing in Ringer would be ineffectual. The oxidizing reserve is not fully exhausted until after 10 to 16 or more hours anoxia (at 20-23°C.), at which time Ringer no longer gives any recovery though oxygen is still able to restore the nerve almost completely.

Earlier workers noting the action of Ringer on asphyxiated nerve (with intact sheath!) have attributed this to a washing out of metabolites, (2) above; but possibly (3) an alteration of the nerve condition must also be considered. The addition of considerable sodium lactate to Ringer does not lessen its restorative action but a much smaller quantity of lactic acid, due to the resultant acidity, does. Carbon dioxide appears to slow the reactions of nerve. Resting nerve produces lactic acid (and no other acid in significant amounts) during anoxia; but this cannot be removed in oxygen, whereas a nerve may fully recover from asphyxia in oxygen alone. Against an outdiffusion of metabolites is the effectiveness of very small quantities of Ringer, just enough to remoisten the nerve surface. On the other hand, the action of Ringer cannot be due to some specific ion, other than Na^+ or Cl^-, since 0.2% NaCl in isotonic glucose is effective. Some ion effect is suggested by analogy with the phenomenon of reversible inexcitability in muscle, and the bene-

ficial action of Ringer on a nerve that has failed to recover from cold block. Finally nerves soaked in isotonic NaCl for 40 minutes before the start of asphyxia, though still fully recovering in oxygen, no longer show any recovery in Ringer. Nerves soaked in NaCl followed by Ringer behave as if they had been in Ringer throughout.

5121

A Note on the Propagation of the Virus in Experimental Poliomyelitis.*

CLAUS W. JUNGEBLUT AND WILLIAM J. SPRING.

From the Departments of Bacteriology and of Physiology, College of Physicians and Surgeons, Columbia University.

While there is good evidence to show that the virus travels along the axis cylinder of the nerve fibers of the spinal cord, particularly from recent work of Fairbrother and Hurst,[1] dissemination by the cerebrospinal fluid has been invoked in explanation by other authors. (Flexner and associates.[2, 3]) A crucial experiment to settle the question would be found in an attempt to recover virus from the cervical cord and from the lumbar cord of a monkey, inoculated intracerebrally, after complete transsection of the spinal cord above the lumbar region. Incidentally, further information might be adduced by histological examination of the 2 separate cord sections for the presence or absence of specific lesions.

Accordingly, we have carried out a complete transsection of the spinal cord in 2 monkeys, severing the structure at the level of the first lumbar vertebra. In the first animal (Monkey 144), 2 silk ligatures were tied tightly around the unopened dural sac, crushing the cord. The whole structure was then cut between the ligatures, the stumps retracting about 1 cm. apart. In the second animal (Monkey 165), the dural sac was opened, but not severed, and the cord was totally divided, leaving a gap of about 3 mm. The operative procedure in the first animal was adopted in order to test the possibility of dissemination by routes other than either the nervous substance or the cerebrospinal fluid, such as the general circulation or the lymphatics. In the second animal our main interest lay in dis-

* Under a grant from the Milbank International Fund for the study of infantile paralysis.

1 Fairbrother, R. W., and Hurst, E. W., *J. Exp. Path. a. Bact.*, 1930, xxxii, 17.

2 Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1914, xx, 249.

3 Amoss, H. L., in Rivers, Th.: Filterable Viruses, Baltimore, 1928.

tinguishing between transmission by the cerebrospinal fluid and through the cord itself.

The 2 transsected monkeys, which immediately after the operation showed a complete paraplegia of the lower extremities, including paralysis of the bladder and rectum, were infected intracerebrally the following day, together with 2 normal controls. (1 cc. of the supernatant fluid of a centrifuged 20% virus cord emulsion.) The first animal died on the eighth day after the inoculation with somewhat indefinite symptoms, though strongly suggestive of poliomyelitis. The upper and the lower cord were removed with care to prevent mutual contamination with virus. Post mortem autolysis, in this case, unfortunately made a conclusive tissue diagnosis impossible. Transfers of the 2 cord sections, respectively, to 2 new monkeys were both negative. The second transsected monkey, which incidentally had suffered much less from traumatic shock than the first, developed on the seventh day all the typical symptoms of poliomyelitis, including tremor and progressive paralysis of the muscles of the arms and the back. It was killed on the ninth day at the height of the infection, with fully developed symptoms, and the upper and lower cord were removed with the same technical precautions. The transfer of the cervical cord to another monkey resulted in typical poliomyelitis in that animal, while the transfer of the lumbar cord was negative. Histological examination showed typical lesions (nerve cell degeneration, neuronophagia, extensive perivascular infiltration and edema) in the cervical and thoracic cord. The lumbar cord appeared normal.

Since, in the normal animal, the lumbar cord represents the place of predilection for the localization of the virus, the absence of virus and of lesions in that structure after transection together with the presence of both in the cervical cord of Monkey 165, are of singular importance. As indicated before, there was full communication of spinal fluid in that animal between the severed cord fragments. While we do not wish to over-emphasize the significance of an isolated observation, the nature of the experiment would make the conclusion almost inevitable that the virus, ordinarily, travels along the nerve tracts. We believe that our results furnish additional evidence for the correctness of the viewpoint of Fairbrother and Hurst on the mode of propagation of the virus in the central nervous system. This theory is further corroborated by the rare occurrence of the virus in the cerebrospinal fluid and by the fact that, in almost all cases, the contralateral limb is the first to show paralysis after intracerebral infection of one hemisphere, an observation which we can fully confirm from our own experience. Our experiments, of

course, have no bearing on the question as to how the virus is disseminated in the body under natural conditions of invasion from the periphery, before it has come in direct contact with the central nervous system.

5122

Feathers as Indicators of Concentration of Female Hormone in the Blood.*

MARY JUHN, G. H. FAULKNER AND R. G. GUSTAVSON.
(Introduced by F. R. Lillie.)

From the Whitman Laboratory of Experimental Zoology and the Department of Physiological Chemistry, University of Chicago.

In the first observations made in this laboratory on the induction of ♀ feathering in capons and cocks subsequent to injections of chemically prepared ♀ hormone during the period of feather regeneration, regional variations in the degree of plumage responses became evident. It was noted that feathers having a more rapid growth rate required definitely higher concentrations of hormone for the female reaction than feathers having a slower growth rate.[1] The growth rates of feathers in various parts of the body have now been accurately measured, and constant regional differences have been established. The birds used for these experiments were brown leghorn fowls, and in this breed the capon is similar in plumage to the cock. In both cock and capon, the growth rate of the feathers is greater in the breast than in the saddle or back, and even greater in the posterior than in the anterior region of the breast. The ratio between the most rapidly and the most slowly growing feathers when measured in growth in length per day is approximately 2:1. In the hen differences in growth rate in various parts of the body are slight, and the general rate for all is intermediate between the maximum and minimum rates of the cock. The threshold concentrations of hormone required for the ♀ reaction are closely correlated with this observed difference in growth rate.

Feathers formed on a capon receiving regular effective daily injections of female hormone are completely female in character; if, however, the injections are restricted to short periods of time, then

* The expenses of this investigation were supported in part by the Committee for Research in Problems of Sex of the National Research Council; grant administered by F. R. Lillie.

[1] Juhn and Gustavson, *J. Exp. Zool.*, 1930, lvi, 31.

bars or patches of female color are laid down on an otherwise male type of feather. One single injection will, if sufficiently large to produce the threshold concentration, be recorded by the feather. If injections are given intermittently, then feathers with alternate bars of female and of male type are formed, the width of the bars varying with the size, and the number, of the injections. On the long saddle feathers it is possible to produce 5 such bars, by a treatment consisting of a sequence of 3 daily injections, then an interval of 3 days with no injections, and a repetition of this cycle 4 times.

In any one bird the induced ♀ bars are broader in the saddle than in the breast feathers, and since the rate of growth *of the female bar* is approximately the same in both, this indicates that the concentration of hormone in the blood was above the saddle threshold for a longer time than it was above the breast threshold: *i. e.*, the more slowly growing saddle feathers have a lower threshold, as indicated above. By measuring the width of the ♀ bars induced in the various types of feather on any one bird in hours of growth, a record is obtained of the relative times at which the concentration of hormone in the blood passed their respective thresholds in its rise and fall.

As the concentration of hormone following injection rises and falls, it passes the thresholds for the various types of feathers in sequence. If one now plots a curve of the cubes of linear growth rates of feathers in the various regions of the body against time it is found that the various thresholds appear on this curve in their natural order and proportions. Such a curve, therefore, expresses the concentration of hormone in the blood, or the affected feather germs, as it rises and falls after injection and cessation of injection, measured in threshold values.

The following additional data for the construction of this curve have been determined by subsidiary experiments. Female pigment is laid down in the feathers growing on the breast 48 hours after injection of an effective dose of the hormone given in olive oil. If the injection is not repeated, the hormone is gradually excreted after this time, but other feathers show that some probably persists in the blood for 72 hours. From this it follows that if daily injections are given, the concentration induced by the first 2 or 3 is a summation, and the maximum attainable by the dosage being used is not reached for about 3 days. If daily injections are omitted on one occasion, the breast feathers with high growth rate and threshold revert during this time to the male type, though the saddle feathers with a lower growth rate and threshold do not. However, if the injections are omitted on 2 consecutive days, the posterior saddle

feathers (growing slightly faster than the anterior saddle) also revert to the male type, and after a 3-day omission the most slowly growing anterior saddle feathers also revert. These observations give an indication of the rate of excretion of the hormone after cessation of injection. They demonstrate that, with the particular preparations and dosages used, the hormone is not entirely excreted in 3 days.

SECRETARY'S ANNUAL REPORT
April 1, 1929 to April 1, 1930

Membership

Members, March 31, 1929	977	
Elected during year	58	
Honorary Members	9	
Total		1044
Resignations, year ending March 31, 1930	7	
Deaths	8	
Dropped for arrears	3	
		18
Net Membership		1026

	1924	1925	1926	1927	1928	1929	1930
Membership	627	692	779	851	936	986	1026

Subscriptions

Subscriptions, March 31, 1929	352	
Subscriptions, March 31, 1930		377
Free subscriptions, March 31, 1929	7	
Free subscriptions, March 31, 1930		8
Exchange subscriptions, March 31, 1929	6	
Exchange subscriptions, March 31, 1930		8
Total, March 31, 1930		393
Total No. of Proceedings distributed 3/31/29		1351
Total No. of Proceedings distributed 3/31/30		1419

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Vice-President: D. J. Edwards.

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1906-07	S. Flexner	E. K. Dunham	W. J. Gies	G. N. Calkins
1907-08	S. Flexner	T. H. Morgan	W. J. Gies	G. N. Calkins
1908-09	F. S. Lee	T. H. Morgan	W. J. Gies	G. Lusk
1909-10	F. S. Lee	W. J. Gies	E. L. Opie	G. Lusk
1910-11	T. H. Morgan	W. J. Gies	E. L. Opie	G. Lusk
1911-12	T. H. Morgan	P. A. Levene	G. B. Wallace	G. Lusk
1912-13	J. Ewing	P. A. Levene	G. B. Wallace	C. Norris
1913-14	J. Ewing	C. W. Field	H. C. Jackson	C. Norris
1914-15	G. Lusk	W. J. Gies	H. C. Jackson	J. R. Murlin
				Secy.-Treas.
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1916-17	J. Loeb	W. J. Gies		H. C. Jackson
1917-18	W. J. Gies	J. Auer		H. C. Jackson
1918-19	W. J. Gies	J. Auer		H. C. Jackson
1919-20	G. N. Calkins	G. B. Wallace		H. C. Jackson
1920-21	G. N. Calkins	G. B. Wallace		H. C. Jackson
1921-22	G. B. Wallace	J. W. Jobling		H. C. Jackson
1922-23	G. B. Wallace	J. W. Jobling		H. C. Jackson
1923-24	H. C. Jackson	J. W. Jobling		V. C. Myers
1924-25	H. C. Jackson	J. W. Jobling		A. J. Goldforb
1925-26	J. W. Jobling	S. R. Benedict		A. J. Goldforb
1926-27	J. W. Jobling	S. R. Benedict		A. J. Goldforb
1927-28	S. R. Benedict	P. Rous		A. J. Goldforb
1928-29	S. R. Benedict	P. Rous		A. J. Goldforb
1929-30	P. Rous	D. Marine		A. J. Goldforb

SECTIONS

Illinois.

Chairman: W. F. Petersen. Secretary: Lloyd Arnold. Members: 82.
Meetings: Chicago Medical Society, November 27, 1929.
University of Illinois, January 21, 1930.
Northwestern University Medical School, February 25, 1930.
Michael Reese Hospital, April 22, 1930.
Billings Hospital, May 27, 1930.

Iowa.

Chairman: F. M. Smith. Secretary: H. M. Hines. Members: 29.
Meetings: State University of Iowa, November 7, 1929.
State University of Iowa, February 7, 1930.
State University of Iowa, April 24, 1930.

Minnesota.

Chairman and Secretary: F. H. Scott. Members: 47.
Meetings: University of Minnesota Medical School, October 30, 1929.
University of Minnesota Medical School, January 29, 1930.
University of Minnesota Medical School, February 26, 1930.
University of Minnesota Medical School, April 30, 1930.
University of Minnesota Medical School, May 28, 1930.

Missouri.

Chairman: P. A. Shaffer. Secretary: M. S. Fleisher. Members: 44.
Meetings: Washington University School of Medicine, November 13, 1929.
St. Louis University School of Medicine, December 11, 1929.
Washington University School of Medicine, February 12, 1930.
St. Louis University School of Medicine, March 12, 1930.
Washington University School of Medicine, April 9, 1930.
St. Louis University School of Medicine, May 14, 1930.

New York.

Chairman: Peyton Rous. Secretary: A. J. Goldforb. Members: 640.
Meetings: New York Academy of Medicine, October 16, 1929.
New York Academy of Medicine, November 20, 1929.
New York Academy of Medicine, December 18, 1929.
New York Academy of Medicine, January 15, 1930.
New York Academy of Medicine, February 19, 1930.
New York Academy of Medicine, March 19, 1930.
New York Academy of Medicine, April 16, 1930.
New York Academy of Medicine, May 21, 1930.

Pacific Coast.

Chairman: W. Ophüls. Secretary: T. D. Beckwith. Members: 80.
Meetings: Stanford University, October 19, 1929.
University of California School of Medicine, December 11, 1929.
Stanford University School of Medicine, February 12, 1930.
University of California, April 12, 1930.

Peiping (China).

Chairman: A. B. D. Fortuyn. Secretary: Hsien Wu. Members: 21.
Meetings: Peiping Union Medical College, November 21, 1929.
Peiping Union Medical College, April 17, 1930.

Southern.

Chairman: H. Laurens. Secretary: W. H. Harris. Members: 25.
Meetings: Tulane University, October 25, 1929.
Tulane University, March 2, 1930.
Tulane University, May 24, 1930.

Western New York.

Chairman: C. F. Cori. Secretary: F. R. Griffith, Jr. Members: 42.
Meetings: Clifton Springs Sanitarium, December 14, 1929.
University of Buffalo Medical School, February 15, 1930.
Cornell University, May 24, 1930.

Wisconsin.

Chairman: L. J. Cole. Secretary: F. L. Hisaw. Members: 20.

DEATHS OF MEMBERS.

The Council records with regret the deaths of the following members: J. F. Cowan, J. A. Harris, Paul A. Lewis, Lillian Moore, and C. J. V. Pettibone.

GIFTS.

The Secretary wishes to acknowledge, with the thanks of the Council, the gift of back numbers from A. R. Dochez.

PROCEEDINGS.

Average length of papers, 1.8 pp.
Preliminary manuscripts, 64%; complete manuscripts, 36%.

A. J. GOLDFORB,
Secretary.

TREASURER'S REPORT
April 1, 1929 to April 1, 1930

Receipts

Balance on hand, April 1, 1929		$ 4,086.22
Income, 1929-30:		
Dues	$ 4,414.10	
Reprints	1,933.59	
Excess Space	1,771.38	
Cuts	345.91	
Subscriptions	2,262.62	
Back numbers sold	350.34	
Bank Interest and Miscellaneous	126.47	
Total Annual Income		$11,204.41
Special Receipts—		
Deposit Account, Peiping Union Medical College		350.00
Total Cash Available, April 1, 1929-April 1, 1930		$15,640.63

Disbursements

Publication Cost of Proceedings:		
Printing	$ 4,518.36	
Reprints	2,470.23	
Cuts	550.67	
		$ 7,539.26
Administrative Expenses:		
Office Supplies, Postage and Telegrams	$ 555.27	
Salaries	2,050.00	
Storage and Insurance	51.75	
Miscellaneous	21.17	
Overpaid	11.50	
		$ 2,689.69
Total Annual Disbursements		10,228.95
Transferred to Special Surplus Account		2,000.00
Cash Balance, April 1, 1930		3,411.68
Total		$15,640.63

Summary

Income (net)	$11,204.41
Disbursements (net)	10,228.95
Surplus for year	$ 975.46

Funds

Endowment Fund

April 1, 1929	$11,170.75
Contributions to April 1, 1930	31.50
*Interest to April 1, 1930	804.17
Total	$12,006.42

* Includes interest on Endowment and on special Surplus Funds.

Invested in New York Title and Mortgage Co.....$ 6,000.00
Invested in R. R. Coop. Bldg. & Loan Assn..... 6,006.42
$12,006.42

Special Surplus Account

Invested in Title Guarantee and Trust Co.....$ 4,500.00

Life Membership Fund

Invested in R. R. Coop. Bldg. and Loan Assn.....$ 75.00

MEMO

Bills payable:
Unexpended balance of Peiping Union Med. College account....$ 13.74
Bills receivable .....$ 1,367.86

A. J. GOLDFORB,
Treasurer.

COMPARATIVE STATEMENT

	1924-25	1925-26	1926-27	1927-28	1928-29	1929-30
Receipts:						
Dues	$2,900.00	$3,336.00	$3,305.00	$ 4,814.95[2]	$ 5,270.93	$ 4,414.10[1]
Reprints	1,300.00	1,381.00	2,039.00	2,174.07	1,996.85	1,933.59
Excess Space	650.00	927.00	1,518.00	1,734.27	1,732.25	1,771.38
Cuts	.....	310.00	308.00	547.75	417.59	345.91
Subscriptions	900.00	1,286.00	1,228.00	2,538.06	2,460.36	2,262.62
Back Numbers	.....	329.00	377.00	571.95	429.25	350.34
Miscellaneous	.....	.....	.....	136.08	144.43	126.47
Total Income	$6,428.00	$7,842.00	$8,880.00	$12,517.13	$12,451.66	$11,204.41
Disbursements:						
Printing }	$4,700.00	$3,353.00	4,113.00[3]	$ 4,253.96	$ 4,048.13	$ 4,518.36
Reprints }		987.00	2,134.00	2,330.96	2,139.14	2,470.23
Cuts	.....	193.00	448.00	577.21	508.33	550.67
Administrative Expense*	2,195.00	2,087.00	2,662.00[5]	3,036.39[4]	3,237.43	2,689.69
Total Disbursements	$6,895.00	$6,623.00	$9,360.00	$10,198.52	$ 9,933.03	$10,228.95
Surplus	—$465.00	—$1,219.00	—$405.00[3,5]	$ 2,318.61[2,4]	$ 2,518.63	$ 975.46[1]

*Includes office supplies, postage, telegrams, salaries, storage, insurance, and miscellaneous.
[1] Dues decreased $1.50.
[2] Dues increased $1.50.
[3] PROCEEDINGS increased by 300 pp.
[4] June number added.
[5] Full time secretary.

Auditors' Report.

We have examined the accounts of the Society, and the Treasurer's report for the year, and have verified the bank balance.

We wish to express our appreciation of the easily understandable system with which the accounts are kept, and the efficient way in which the system is applied under the direction of the Treasurer by Miss McCabe.

(Signed) ARTHUR F. COCA,
JEAN OLIVER.

April 7, 1930.

MEMBERS' LIST (Alphabetical)

Honorary Members

Councilman, William T. ..... Harvard University
Halliburton, W. D. ..... London, England
Lombard, Warren P. ..... University of Michigan
Porter, William ..... Harvard University
Reichert, E. T. ..... University of Pennsylvania
Richet, Charles ..... Paris, France
Schaffer, E. S. ..... Edinburgh, Scotland
Von Muller, Friedrich ..... Munich, Germany
Welch, W. H. ..... Johns Hopkins University

Members

Abbott, Alexander C. ..... University of Pennsylvania
Abel, John J. ..... Johns Hopkins University
Adams, A. Elizabeth ..... Mount Holyoke College
Addis, Thomas ..... Medical School, Stanford University
Adler, Herman M. ..... Santa Barbara, California
Albus, William R. ..... American Type Culture Collection, Chicago
Alexander, Harry L. ..... Washington University
Allen, Bennet M. ..... University of California
Allen, Edgar ..... University of Missouri
Allen, Ezra ..... N. Y. Homeopathic Medical College
Allen, William F. ..... University of Oregon
Alsberg, Carl L. ..... Stanford University
Alvarez, Walter C. ..... Mayo Clinic
Amberg, Samuel ..... Mayo Clinic
Amoss, Harold L. ..... Johns Hopkins Hospital
Anderson, E. G. ..... California Institute of Technology
Anderson, John F. ..... E. R. Squibb & Son
Anderson, Rudolph J. ..... Yale University
Andrews, Edmund ..... University of Chicago
Anson, Mortimer L. ..... Rockefeller Institute, Princeton, N. J.
Arnold, Lloyd ..... University of Illinois
Asher, Leon ..... Berne, Switzerland
Ashman, Richard ..... Tulane University
Atchley, D. W. ..... Presbyterian Hospital, New York City
Atkinson, H. V. ..... Vermilion, S. D.
Atwell, Wayne J. ..... University of Buffalo, N. Y.
Aub, Joseph C. ..... Huntington Memorial Hospital, Boston
Auer, John ..... St. Louis University
Austin, J. Harold ..... University of Pennsylvania
Austin, W. C. ..... Loyola University
Avery, O. T. ..... Rockefeller Institute, N. Y. City

Bachem, Albert ..... University of Illinois Medical College
Baehr, George ..... Mt. Sinai Hospital, N. Y. City

Bagg, Halsey J.....Memorial Hospital, N. Y. City
Bailey, Cameron V.....N. Y. Post-Graduate Medical School
Baitsell, George A.....Yale University
Baldwin, W. Manning.....Albany Medical College
Ball, G. H.....University of California, Los Angeles
Balls, A. K.....Bremen, Germany
Banta, A. M.....Station for Exp. Evolution, Cold Spring Harbor, N. Y.
Banzhaf, Edwin J.....N. Y. Health Department
Barach, Alvan L.....College of Physicians and Surgeons
Barber, W. Howard.....New York University Med. School
Barbour, Henry G.....University of Louisville
Bardeen, Charles R.....University of Wisconsin
Barlow, Orphus W.....Western Reserve University
Barnett, George D.....Stanford University
Barr, David P.....Washington University
Bass, Charles .....Tulane University
Bast, T. H.....University of Wisconsin
Bauer, J. H.....Rockefeller Institute
Bauman, Louis .....Presbyterian Hospital, N. Y. City
Baumann, E. J.....Montefiore Hospital, N. Y. City
Baumberger, J. Percy.....Stanford University
Bayne-Jones, S. .....University of Rochester
Bazett, H. C.....University of Pennsylvania
Beard, H. H.....Western Reserve University
Becker, E. R.....Iowa State College
Becking, L. B.....Jacques-Loeb Lab., Pacific Grove, Calif.
Beckwith, T. D.....University of California
Behre, Jeannette A.....Cornell University Medical College
Belding, David L.....Boston University
Bellamy, Arthur W.....University of California, Los Angeles
Benedict, S. R.....Cornell University Medical College, N. Y. City
Benton, Anne G.....Vassar College
Berg, B. N.....Columbia University
Berg, William N.....Berg Biological Laboratory, N. Y. City
Bergeim, Olaf.....University of Illinois
Bergey, David H.....University of Pennsylvania
Berglund, Hilding.....University of Minnesota
Bernhard, Adolph .....Lenox Hill Hospital, N. Y. City
Beutner, R. .....University of Louisville
Bills, C. E.....Mead, Johnson and Co., Evansville, Ind.
Binger, Carl A. L. .....Rockefeller Institute, N. Y. City
Birkhaug, Konrad E.....University of Rochester
Bishop, George H. .....Clayton, Mo.
Bishop, Katharine S.....University of California
Blackfan, K. D.....Harvard Medical School
Blake, F. G.....Yale University
Blakesley, Albert F.....Station for Exp. Evolution, Cold Spring Harbor, N. Y.
Blatherwick, Norman R.....Metropolitan Life Insurance Co., New York City
Blau, Nathan F.....Cornell University Medical College
Blinks, L. R.....Rockefeller Institute
Bliss, Sidney .....Tulane University
Bloom, William.....University of Chicago
Bloomfield, A. L.....Stanford University Medical School
Bloor, W. R.....University of Rochester
Blum, Harold F.....Harvard Medical School

Blumgart, H. L.....Beth Israel Hospital, Boston
Bodansky, A.....New York City
Bodansky, Meyer.....University of Texas Medical College
Bodine, J. H.....State University of Iowa
Bollman, Jesse L.....Mayo Clinic
Boothby, Walter M.....Kahler Hospital, Rochester, Minn.
Boots, Ralph H.....Rockefeller Hospital
Bowen, B. D.....Buffalo General Hospital
Boyd, Julian D.....State University of Iowa
Boyd, Theo. E.....Loyola University
Boyden, E. A.....University of Alabama Medical School
Bradley, H. C.....University of Wisconsin
Brand, Erwin.....New York State Psychiatric Institute
Branham, Sara E.....Hygienic Laboratory, Washington, D. C.
Brewer, Robert K.....Syracuse University
Briggs, A. P.....St. Louis University
Bronfenbrenner, J.....Washington University
Bronk, D. W.....University of Pennsylvania
Brooks, Clyde.....University of Alabama
Brooks, Harlow.....New York University
Brooks, Matilda M.....University of California
Brooks, S. C.....University of California
Broun, G. O.....St. Mary's Infirmary, St. Louis
Brown, E. D.....University of Minnesota
Brown, J. Howard.....Johns Hopkins University
Brown, John B.....Ohio State University
Brown, L. A.....George Washington University
Brown, Wade H.....Rockefeller Institute, N. Y. City
Buchanan, Robert E.....Iowa State College
Buchbinder, W. C.....Michael Reese Hospital, Chicago
Bulger, H. A.....Washington University
Bull, C. G.....Johns Hopkins University
Bunting, C. H.....University of Wisconsin
Bunting, R. W.....University of Michigan
Burget, G. E.....University of Oregon Medical School
Burnett, Theodore C.....University of California
Burns, Robert K., Jr.....University of Rochester
Burr, Harold S.....Yale University
Burrows, M. T.....Pasadena, Calif.
Burton-Opitz, Russell.....Palisade, N. J.
Byrne, Joseph.....Fordham University

Calkins, Gary N.....Columbia University
Cannon, Paul R.....University of Chicago
Cannon, Walter B.....Harvard Medical School
Carlson, A. J.....University of Chicago
Carmichael, E. B.....University of Alabama School of Medicine
Carpenter, Charles M.....Albany Medical College
Carter, Edward P.....Johns Hopkins Hospital
Cash, James R.....Peiping Union Medical College, China
Castellani, Aldo.....Tulane University
Cattell, McKeen.....Cornell University Medical College
Caulfield, A. H.....University of Toronto
Cecil, R. L.....Cornell University Medical College
Chace, Arthur F.....N. Y. Post-Graduate Medical School

Chambers, Robert ..... New York University
Chambers, William H. ..... Cornell University Medical College
Chang, H. C. ..... Peiping Union Medical College
Cheer, S. N. ..... Peiping Union Medical College, China
Chen, K. K. ..... Eli Lilly and Co., Indianapolis
Chidester, F. E. ..... University of West Virginia
Child, C. M. ..... University of Chicago
Chittenden, R. H. ..... Yale University
Chou, T. ..... Peiping Union Medical College, China
Christian, Henry A. ..... Peter Bent Brigham Hospital
Christman, Adam A. ..... University of Michigan
Churchman, John W. ..... Cornell University Medical School
Clark, Guy W. ..... Lederle Lab., Pearl River, N. Y.
Clark, P. F. ..... University of Wisconsin
Clarke, Hans T. ..... College of Physicians and Surgeons
Claussen, S. W. ..... Strong Memorial Hospital, Rochester, N. Y.
Clowes, G. H. A. ..... Eli Lilly and Co., Indianapolis, Indiana
Coca, A. F. ..... Cornell University Medical College
Coghill, G. E. ..... Wistar Institute
Cohen, Barnett ..... Johns Hopkins Medical School
Cohen, Martin ..... N. Y. Post-Graduate Medical School
Cohn, A. E. ..... Rockefeller Institute, N. Y. City
Cohn, Edwin J. ..... Harvard Medical School
Cohn, Isidore ..... New Orleans, La.
Cole, L. J. ..... University of Wisconsin
Cole, Rufus I. ..... Rockefeller Institute, N. Y. City
Cole, William H. ..... Rutgers University
Coleman, Warren ..... New York University Medical College
Collens, William S. ..... Brooklyn Jewish Hospital
Collett, Mary E. ..... Western Reserve University
Collier, William D. ..... St. Louis University
Collins, H. H. ..... University of Pittsburgh
Collip, J. B. ..... McGill University
Conklin, E. G. ..... Princeton University
Cook, S. F. ..... University of California
Cooke, J. V. ..... Washington University
Coombs, Helen C. ..... New York Homeopathic Medical College
Copenhaver, W. M. ..... Columbia University
Cori, Carl F. ..... State Institute of Malignant Diseases, Buffalo, N. Y.
Corley, Ralph C. ..... Purdue University
Corner, George W. ..... University of Rochester
Corper, H. J. ..... National Jewish Hospital, Denver, Colo.
Coulter, Calvin B. ..... Columbia University
Couret, M. J. ..... Tulane University
Cowdry, E. V. ..... Washington University
Cowgill, George R. ..... Yale University
Crampton, C. Ward ..... N. Y. Post-Graduate Medical School
Crile, George W. ..... Western Reserve University
Crohn, Burrill B. ..... Mt. Sinai Hospital, N. Y. City
Crozier, W. J. ..... Harvard University
Cruickshank, E. W. H. ..... University of Patna, Patna, India
Csonka, F. A. ..... U. S. Bureau Chemistry, Washington, D. C.
Cullen, Glenn E. ..... Vanderbilt University
Cummins, Harold ..... Tulane University
Cunningham, R. S. ..... Vanderbilt University

Curtis, G. M.....University of Chicago
Curtis, Maynie R.....Columbia University
Cushing, Harvey W.....Harvard Medical School
Cutler, Elliott C.....Western Reserve University

Dack, Gail M.....University of Chicago
Dakin, H. D.....Ossining, N. Y.
Danforth, Charles H.....Harvard Medical School
Daniel, J. Frank.....University of California
Daniels, Amy L.....University of Iowa
Daniels, Farrington.....University of Wisconsin
Danzer, Charles S.....N. Y. Homeopathic Medical College
Davenport, C. B.....Sta. for Exp. Evolution, Cold Spring Harbor, N. Y.
Davies, H. Whitridge.....The University, Sydney, Australia
Davis, D. J.....University of Illinois
Davison, Wilburt C.....Duke University
Dawson, James A.....College of the City of New York
Day, A. A.....Northwestern University Medical School
De Eds, Floyd.....Hygienic Lab., Washington, D. C.
DeGraff, A. C.....N. Y. University Medical School
Derick, C. L.....Peter Bent Brigham Hospital, Boston
Detwiler, S. R.....Columbia University
Deuel, Harry J., Jr.....University of Southern California Medical School
Dickson, E. C.....Stanford University Medical School
Dienes, Louis.....Von Ruck Research Lab., Asheville, N. C.
Dieuaide, Francis R.....Peiping Union Medical College, China
Doan, Charles A.....Rockefeller Institute
Dochez, A. R.....Presbyterian Hospital, N. Y. City
Dock, William.....Stanford University
Doisy, Edward A.....St. Louis University
Donaldson, H. H.....Wistar Institute, Philadelphia
Donaldson, J. C.....University of Pittsburgh
Dooley, M. S.....Syracuse University
Drabkin, D. L.....University of Pennsylvania
Dragstedt, Carl A.....Northwestern University
Dragstedt, Lester R.....Northwestern University
Draper, George W.....Columbia University
Draper, John W.....New York City
Dresbach, M.....Albany Medical College
Dubin, Harry E.....Metz Laboratories, N. Y. City
DuBois, E. F.....Cornell University Medical College
Duggar, B. M.....University of Wisconsin
Dunn, Halbert L.....Mayo Clinic
Dunn, L. C.....Columbia University
Dunn, Max.....University of S. California
Dutcher, R. Adams.....Pennsylvania State College
Duval, C. W.....Tulane University
Dye, Joseph A.....Cornell University Medical College

Eberson, Frederick.....University of California Medical School
Ecker, E. E.....Western Reserve University
Eckles, C. H.....University of Minnesota
Eckstein, Henry C.....University of Michigan
Eddy, Walter H.....Columbia University
Edmunds, C. W.....University of Michigan

Edwards, D. J.....Cornell University Medical College
Eggston, Andrew A.
Manhattan Eye, Ear, Nose and Throat Hospital, N. Y. City
Eisberg, Harry B.....N. Y. University and Bellevue Medical College
Ellis, Max M.....University of Missouri
Elsberg, Charles A.....Neurological Institute, N. Y. City
Elser, W. J.....Cornell University Medical College
Engle, E. T.....Columbia University
Epstein, A. A.....Mt. Sinai Hospital, N. Y. City
Erdmann, Rhoda.....University of Berlin, Germany
Erlanger, Joseph .....Washington University
Evans, Herbert M.....University of California
Ewing, James .....Cornell University Medical College
Eyster, J. A. E. .....University of Wisconsin

Faber, Harold K.....Stanford University Medical School
Fahr, George.....University of Minnesota
Falk, I. S.....Washington, D. C.
Falk, K. George.....Harriman Laboratories, N. Y. City
Falls, Frederick H.....University of Illinois
Famulener, L. W.....St. Luke's Hospital, N. Y. City
Farmer, Chester.....Northwestern University
Faust, Ernest C.....Tulane University
Ferry, R. M.....Harvard University
Field, Cyrus W.....New York City
Fine, M. S.....Battle Creek, Mich.
Fischer, Albert .....Berlin, Germany
Fischer, Martin H. .....University of Cincinnati
Fish, Pierre A.....Cornell University
Fishberg, Ella H.....New York City
Fisher, Nelson F.....St. Luke's Hospital, Chicago
Fitch, C. P.....University of Minnesota
Fitz, Reginald .....Peter Bent Brigham Hospital
Fitzgerald, J. G.....University of Toronto
Fleisher, Moyer S.....St. Louis University
Flexner, Simon.....Rockefeller Institute, N. Y. City
Flinn, Frederick B.....Columbia University
Florence, Laura.....New York Homeopathic Medical School
Forbes, Henry.....Milton, Mass.
Forkner, Claude E.....Rockefeller Institute
Fortuyn, A. B. D.....Peiping Union Medical College
Foster, Goodwin L.....Columbia University
Foster, Nellis B.....New York Hospital, N. Y. City
Frank, Robert T.....Mt. Sinai Hospital
Fred, E. B.....University of Wisconsin
Freedman, Louis.....Metz Laboratory
Freund, Jules.....Henry Phipps Institute, Philadelphia
Fridericia, L. S.....University of Copenhagen, Denmark
Frobisher, Martin, Jr.....Rio de Janiero, Brazil
Fry, Henry J.....New York University
Fulmer, Ellis I.....Iowa State College
Fulton, John F.....Oxford, England
Funk, Casimir.....Brussels, Belgium
Furth, Jacob.....Henry Phipps Institute, Philadelphia

Gaebler, O. H.—Henry Ford Hospital, Detroit
Gager, C. Stuart—Brooklyn Botanic Garden, Brooklyn, N. Y.
Gahl, Rudolph—University of California
Gamble, James L.—Harvard University
Garbat, Abraham L.—Lenox Hill Hospital, N. Y. City
Gardner, Leroy U.—Saranac Lab. for Study of Tuberculosis
Garrey, Walter E.—Vanderbilt University
Gaskell, John F.—Cambridge, England
Gasser, Herbert S.—Washington University
Gates, Frederick L.—Cambridge, Mass.
Gay, F. P.—Columbia University
Geiling, E. M. K.—Johns Hopkins Medical School
Gerard, R. W.—University of Chicago
Gesell, Robert A.—University of Michigan
Gettler, A. O.—University and Bellevue Medical College
Geyelin, Henry R.—Columbia University
Gibson, R. B.—University of Iowa
Gies, William J.—Columbia University
Githens, T. S.—Mulford Company, Philadelphia, Pa.
Givens, Maurice H.—Northwestern Yeast Co., Chicago
Glaser, Otto—Amherst College
Gold, Harry—Cornell University Medical College
Goldberg, S. A.—Bronx Hospital, N. Y.
Goldblatt, Harry—Western Reserve University
Goldbloom, A. Allen—Beth Israel Hospital, N. Y. City
Goldforb, A. J.—College of the City of New York
Goldschmidt, Samuel—University of Pennsylvania
Gortner, R. A.—University of Minnesota
Gottesman, J. Marmorston—Montefiore Hospital, N. Y. City
Gould, Harley N.—Tulane University
Graham, Evarts A.—Washington University
Graves, William W.—St. Louis University
Gray, Samuel H.—Jewish Hospital, St. Louis, Mo.
Green, Robert G.—University of Minnesota
Greenberg, David N.—University of California
Greene, Carl H.—Mayo Clinic
Greenwald, Isidor—Littauer Laboratory, N. Y. City
Greer, J. R.—University of Chicago
Gregory, Louise H.—Barnard College, Columbia University
Greisheimer, Esther M.—University of Minnesota
Griffith, Fred R., Jr.—University of Buffalo
Griffith, Wendell H.—St. Louis University
Gross, Erwin G.—State University of Iowa
Gross, Louis—Mt. Sinai Hospital, N. Y. City
Gruber, Charles M.—Washington University
Guberlet, J. E.—University of Washington
Guenther, A. E.—University of Nebraska
Gurchot, Charles—Stanford University Medical School
Guthrie, C. C.—University of Pittsburgh
Guy, Ruth A.—Peiping Union Medical College
Guyer, Michael F.—University of Wisconsin

Hadley, Philip—University of Michigan
Hafkesbring, H. Roberta—Tulane University
Hagan, William Arthur—Cornell University

Hale, Worth.....Harvard Medical School
Hall, C. Ivan.....University of Colorado
Halsey, John.....Tulane University
Halsey, Robert H.....N. Y. Post-Graduate Medical School
Hamburger, W. W.....Rush Medical College
Hamilton, B. K.....Baltimore, Md.
Hammer, B. W.....Iowa State College
Hanke, Martin E.....University of Chicago
Hannon, R. R.....Rockefeller Institute
Hansman, G. H.....State University of Iowa
Hanson, Frank B.....Washington University
Hanzlik, P. J.....Stanford University Medical School
Hardesty, Irving.....Tulane University
Harris, Isaac F.....Tuckahoe, N. Y.
Harris, Reginald G.....Cold Spring Harbor, N. Y.
Harris, William H.....Tulane University
Harrison, R. G.....Yale University
Harrop, George A., Jr.....Johns Hopkins University
Harrow, Benjamin.....College of the City of New York
Hartman, F. A.....University of Buffalo
Hartwell, John A.....Cornell University Medical College
Harvey, E. Newton.....Princeton University
Harvey, Samuel C.....Yale University
Hastings, A. Baird.....University of Chicago
Hastings, E. G.....University of Wisconsin
Hatcher, R. A.....Cornell University Medical College
Hathaway, Edward S.....Tulane University
Hawk, P. B.....New York City
Hawkins, James A.....Rockefeller Institute
Hayden, Charles E.....Cornell University
Haythorn, Samuel R.....Singer Research Lab., Pittsburgh, Pa.
Heft, Hattie L.....Columbia University
Heidelberger, Michael.....Presbyterian Hospital, New York City
Heilbrunn, L. V.....University of Pennsylvania
Helff, O. M.....New York University
Helmholz, Henry R.....Mayo Clinic
Hemmeter, John C.....Baltimore, Md.
Hench, Philip S.....Mayo Clinic
Hendrix, B. M.....University of Texas
Henrici, Arthur T.....University of Minnesota
Herrmann, G. R.....Tulane University
Hertzman, A. B.....St. Louis University
Hess, Alfred F.....N. Y. University and Bellevue Medical School
Heymans, J. F.....U. de Gand, Belgium
Higgins, George M.....Mayo Clinic
Hill, Eben C.....Johns Hopkins University
Hill, Samuel E.....Rockefeller Institute
Hiller, Friedrich.....University of Chicago
Himwich, H. E.....Yale University
Hines, H. M.....State University of Iowa
Hinrichs, Marie Agnes.....University of Chicago
Hinshaw, H. C.....American University of Beirut, Syria
Hirschfelder, Arthur.....University of Minnesota
Hisaw, F. L.....University of Wisconsin
Hitchcock, Fred A.....Ohio State University

Hoffman, George L.....Allegheny County Hospital, Pittsburgh, Pa.
Holm, George E.....Department of Agriculture, Washington, D. C.
Holman, Emil .....Stanford University
Holman, W. L.....University of Toronto
Holmes, S. J.....University of California
Holt, L. Emmett, Jr.....Johns Hopkins University
Hooker, Davenport.....University of Pittsburgh
Hooker, Sanford B.....Boston University
Hooper, Charles W.....Metz Laboratories
Hopkins, J. Gardner.....Columbia University
Horsley, J. Shelton.....St. Elizabeth's Hospital, Richmond, Va.
Howard, Harvey J.....Washington University
Howe, Paul E.....Department of Agriculture, Washington, D. C.
Howell, K. M.....Michael Reese Hospital
Howell, William H.....Johns Hopkins University
Howland, Ruth B.....New York University
Hubbard, Roger S.....Clifton Springs Sanitarium, N. Y.
Huber, G. Carl.....University of Michigan
Huestis, R. R.....University of Oregon
Hunt, Reid .....Harvard University
Huntoon, F. M.....Mulford Co., Glenolden, Pa.
Hurwitz, Samuel.....Stanford Medical School
Hussey, Raymond .....Yale University

Irving, Lawrence .....University of Toronto
Irwin, Marion.....Rockefeller Institute
Isaacs, Raphael.....Simpson Memorial Institute, Ann Arbor
Ivy, Andrew C.....Northwestern University

Jackson, C. M.....University of Minnesota
Jackson, D. E.....University of Cincinnati
Jackson, Henry, Jr.....Boston City Hospital
Jacobs, M. H.....University of Pennsylvania
Jacobs, Walter A.....Rockefeller Institute, N. Y. City
Jacobsen, Victor C.....Albany Medical College
Jaffe, Henry L.....Hospital for Joint Diseases, N. Y. City
Jaffe, Richard H.....University of Illinois
Jeans, Philip C.....University of Iowa
Jennings, H. S.....Johns Hopkins University
Jobling, J. W.....Columbia University
Johlin, J. M.....Vanderbilt University School of Medicine
Johns, Foster M.....Tulane University
Johnson, C. A.....University of Chicago
Johnson, C. C.....San Francisco, Calif.
Johnson, T. B.....Yale University
Jonas, Leon .....University of Pennsylvania
Jones, Frederick S.....Rockefeller Institute, Princeton, N. J.
Jordan, Edwin .....University of Chicago
Jordan, H. E.....University of Virginia
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AUTHORS' INDEX

(The numerals indicate the page.)

SUBJECT INDEX

(*The numerals indicate the page.*)